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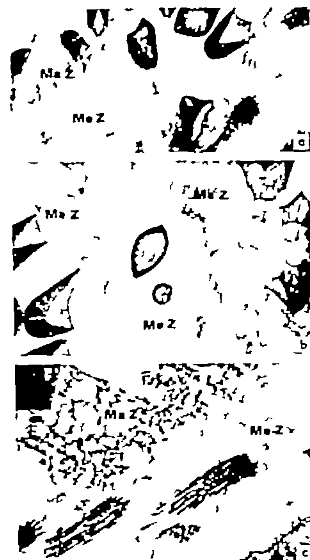


Fig 1 (*a*) Section through the otolithic utricular membrane of the guinea pig. The two zones of different electron density and a wavy arrangement are clearly seen. The otoliths are partially immersed in the marginal zone (*MaZ*). The macular epithelium and sensory hair bundles are located at the bottom right. Medial zone (*MeZ*). 9_5 (*b*) Otoliths ppear total or partially immersed in the otolithic membrane. Most of otoliths are lying on the marginal zone (*MaZ*). × 1 850 (*c*) Oblique section of the otolithic membrane showing its two zones. Various sensory hair bundles in the medial zone, and otoliths in the marginal zone can be found. The sensory epithelium is seen at the lower right corner 1 850.

MT 1 ultramicrotome and stained with lead citrate (Reynolds, 1963). They were examined with a Siemens Elmiskop I electron microscope at 60 Kv Photographs were taken on 23 d 50 Gevaert Scientia films with initial magnifications ranging from 1 000 to 40 000 ×

Findings

Otolithic membrane

The otolithic membrane is situated on the sensorial epithelium showing a wavy free surface. It consists of two distinct zones of different electron density one marginal and the other medial. Of these, the latter is less dense than the former

The otoliths can be seen in the marginal zone and the sensory hair bundles in the medial, although occasionally otoliths appear in the medial zone (Fig. 1 *b*)

(*a*) *Marginal Zone* (*MaZ*) We named it so because it is situated further from the sensorial epithelium. It consists of a fibrous network and an amorphous interfibrillar substance The otoliths appear to be partially immersed in the marginal zone forming a first stratum Otoliths of the further layers probably rest on prolongations of the otolithic membrane. Occasionally in certain parts of the first stratum and in proximity to the otoliths it is possible to observe granular or vesicular structures. They are spherical or irregular in shape enclosing a substance of similar or greater electronic density than that of the otolithic membrane (Fig. 3 *b*)

(*b*) *Medial Zone* (*MeZ*) This is the part of the otolithic membrane near the sensorial epithelium. The limits which separate it from the marginal zone are imprecise for both structures. They are very similar differing only in that the medial zone contains less amorphous interfibrillar substance and its fibrous network is more loose We have observed no subcupular space, because the fibrillar material is close to and enters in contact with the apical surface of the sensorial and supporting cells (Fig. 2 *a*) Generally in the striola this zone is more dense (Fig. 2 *b*)

Sensory hair bundles and some otoliths can be seen within this zone We have not found a

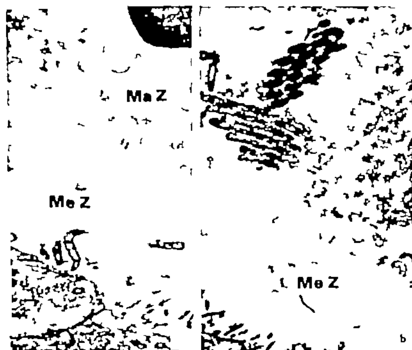


Fig (*a*) Marginal (*MaZ*) and Medial (*MeZ*) Zones of the otolithic membrane. The latter reaches the sensory epithelium and no subcupular space can be detected. 10 000. (*b*) Striola showing tw bundles crossing at different angles to each other Also at this point the medial zone (*MeZ*) reaches the epithelium and show condensation near the crossing sensory hair bundles. 10 000.

direct connection between the otoliths and cilia.

Otoliths

They usually have a cylindrical body with faceted and pointed extremities. Their length ranges approximately from 6 to 20 μm.

(*a*) *Body* Round or oval in shape, it appears to consist of two areas of different electronic density· a very dense one at the periphery and a clear one at the centre.

The central area consists of two parts, an inner and an intermediate, both differing in density The former consists of a material similar to that of the otolithic membrane medial zone (MeZ) However the density of the latter is reminiscent of that of the marginal zone (MaZ) (Fig. 1 *b*) Both parts differ also in the arrangement of their constituent material. The intermediate part presents a filamentous structure whose components are oriented parallel to the main axis of the otolith. The thickness of the filaments ranges from 50 to 70 Å. In the inner part a wide spaced filamentous network can be observed (Fig. 4 *a*)

The peripheral area is generally very dense, but it contains zones similar in their appearence to the intermediate part of the central area. The elementary components are granules having a diameter between 80 to 140 Å.

The limits between this and the central area are irregular since the material of one overlaps and mixes with that of the other In the parts close to the extremities of the otolith, the dark material penetrates almost to the centre (juxtamarginal condensation)

In the bodies of some otoliths it is possible at times to distinguish fissures recalling images of fracture lines. They divide the dense peripheral zone in several fragments. The otolithic membrane material appears to penetrate through them (Fig. 3 *a*)

(*b*) *Extremities* These are pyramidal in shape, showing several flat or hollow facets.

The extremity itself consists of a low density filamentous-like substance showing a perpendicular arrangement to the surface facet. The inner limits of this material are formed by a sharp line parallel to the facet surface line. Both surfaces consist of a layer of dense gran-

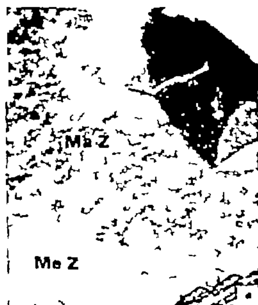




Fig 3 ( ) Otolith showing fissure in its body Material of the dense marginal zone of the otolithic membrane enters in this fissure (*arrow*). At the bottom right, a sensory hair bundle can be seen. 10 000.

(*b*) Membranous structures (possibly cellular debris) partially immersed in the marginal zone of the otolithic membrane. 15 000.

ules. Those of the facet surface measure between 80–120 A in diameter Those of the inner limiting surface vary between 60–80 A n diameter (Fig. 4 *a*) The angles formed between facet and body surface usually measure 00–150 However the angles formed between the facet surfaces varies from 90 to 110

Otoliths with irregular facets

Their general arrangement is similar to those already described. However they have more irregular facet surfaces and their bodies show a greater number of fissures (Fig. 4 *b*) The otolithic membrane material appears to penetrate through them

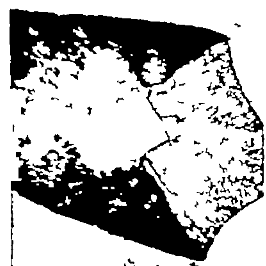




Fig 4 (*a*) Oblique section of an otolith showing zones of different electron density and arrangement of filamentous material of its facets and body 12 500.

(*b*) Otolith presenting several body fissures (*arrow*). Its facets have an irregular conformation. 7 500.



Fig. 5 (*a*) Ground substance of the otolithic membrane connecting two otoliths (*arrows*). 15 000. (*b*) The otolithic membrane material connecting three otoliths shows an apparent continuity with the body light areas of the centrally placed otolith 20 000.

Relations between the otoliths

Occasionally connections between two or more neighbouring otoliths can be observed. The connections may be as follows: (*a*) facet to facet, (*b*) body to body (*c*) facet to body

Usually a strip of the otolithic membrane material can be observed between contact surfaces (Fig. 5 *a*) On the other hand, intimate contact suggesting fusion zones can also be found.

It is occasionally observed that the otolithic membrane material connecting two or more otoliths seems to continue with the body light areas of one of them (Fig. 5 *b*).

DISCUSSION

Otolithic membrane

The otolithic membrane was defined by Retzius as a "gelatinous layer" Polyak et al., 1946 described it as "a jelly-like substance, of almost homogeneous structure, in which the hairs of the hair cells are embedded" Igarashi (1966), in his work on the squirrel monkey states that "The otolithic zone itself is almost flat, except for some wavy formations, specially at the edge. At these wavy points some spaces between the otolithic and cupular zones could be observed

We have seen that the otolithic membrane has a wavy appearance, but does not show any empty space below the elevation. Igarashi & Kanda (1969) distinguish in the otolithic membrane of the squirrel monkey two zones which they denominate "cupular zone 1" and "cupular zone 2" These are the same zones observed by us and called marginal and medial zones, respectively

Vilstrup (1950) Wersäll (1968) Igarashi & Kanda (1969) discovered a subcupular space whose content is not known. On the other hand, we have observed that the medial zone of the otolithic membrane continues up to the sensorial epithelium, although in this area the

filamentous network is very lax. This fact has also been confirmed by us in the striola. These findings were also communicated by Kolmer (1927) and by Lindeman (1969 *a*) in the guinea pig. Moreover Lindeman (1969 *a*) found that in the striola there are "broad clearings in the gelatinous substance"

According to our observations the medial zone contains sensory hair bundles and isolated otoliths, whereas in the marginal zone there are only otoliths partially or totally embedded in it. This opinion is shared by Lim (1969) as opposed to Johnsson & Hawkins (1967) who express the opinion that in the human the otoliths tend to adhere to the superior surface of the otolithic membrane. According to Kellerhals et al. (1970) the otoliths are embedded in the gelatinous ground substance of the central region of the utricular macula.

We have not found a direct connection between the otoliths and cilia observed by Iurato & De Petris (1967) and demonstrated by them in the rat.

The otolithic membrane was described as early as 1925 by Tenaglia. His findings were [illegible] by Brock (1926) in the bird, by [illegible]ittmaack (1956) in the guinea pig, rabbit and dog, by Johnsson & Hawkins (1967) in the human, and by Lindeman (1967 1969 *a*, 1969 *b*) in the guinea pig. Spoendlin (1964) believes that its fibrillar appearance may be an artefact. In our material the presence of filaments has been confirmed. Ground substance presenting a greater density in the marginal zone and in some areas of the medial zone (in the striola) as described by Werner (1933) and by Lindeman (1969 *a*) can also be found.

Otoliths

Carlström & Engström (1955) studied human otoliths by means of electron microscopy and X-ray diffraction. They pointed out that these are formed by small hexagonal platelets so well aligned that the whole otolith behaves in X ray diffraction as a single crystal of calcite. In most of the images offered by these authors, the otoliths appear to be formed by a very dense structure which does not permit the passage of electrons.

The images presented by Iurato & De Petris (1967) are also similar Igarashi & Kanda (1969) obtained electron microphotographs of high quality permitting the observance of zones with different electronic density. As a result of such findings these authors stated that the electronic density is very irregularly distributed. Sugiura & Hilding (1970) presented electron micrographs of otoliths in the adult Hedlund mink. However these pictures do not clearly demonstrate its constitution.

Barber & Boyde (1968) were the first to describe the calcareous statolith of Eledone as a cone-shaped structure by means of the scanning electron microscope (S.E.M.) Afterwards Lim (1969), Lim & Lane (1969) and Kellerhals et al. (1970) studied the otoliths of the guinea pig and showed that they have cylindrical bodies with two pointed extremities.

Kolmer (1927) describes a nucleus in the otoliths in which he postulates the initial crystal formation. Lindeman (1969 *a*) in the guinea pig confirmed the existence of such a nucleus which we believe corresponds to the clearer central area.

In our material we confirm the existence of zones of different electronic density. Generally the denser zones are situated in the peripheral part of the cylindrical surface. The lighter ones are situated at the centre and on the lateral facets. Their constituent filamentous material is always arranged perpendicular to the facet surface. However in the body the filaments are parallel to their main axis. In certain aspects the arrangement of this material recalls the organization of the dental enamel inorganic crystals of bovine embryos (Glimcher et al 1965).

At present we are carrying out microdiffractographic studies (Sánchez Fernández & Rivera-Pomar 1970) in order to confirm the nature and arrangement of the material within the light and dark zones. Hitherto these studies seem to indicate that both have a mic-

rocrystalline constitution. We believe that differences in density between the otolith centre and periphery may be due to their unequal content of calcium salts.

Concerning the genesis of the otoliths, Vilstrup (1951) in the shark indicates that they are formed within the epithelial cells of the endolymphatic sac, and migrate posteriorly to the lumen. De Vincentiis & Marmo (1966) suggested that otolith development in the chick takes place in two stages: 1) An initial phase characterized by *formation of the organic matrix* which consists of mucopolysaccharides and proteins (this matrix is secreted in certain zones of the membranous labyrinth endolymphatic sac and macular zone) 2) a phase of mineralization characterized by the deposition of calcium carbonate in the organic matrix" Recently Balsamo et al. (1969) in the chick confirm the hypothesis of the active participation of the endolymphatic sac in the morphogenesis of the otoliths. They also claim that these are formed by a fusion and accretion process, and we are in agreement with them about this last possibility

However the apparent morphological identity between central areas of the otolith body and the two zones of the otolithic membrane, as well as the fact that the otolithic membrane material penetrates through the otolith body fissures, lead us to believe that the otoliths are generated within the otolithic membrane. Perhaps the ionic component of the utricular endolymph and the content of the saccular structures, seen by us near the otoliths, play an important role in modelling them.

RESUME

Nous vons étudié l'ultrastructur de la membrane otolithique de la macula utriculaire du cobay et observé *qu elle adopte une position ondulée et qu elle* est formée par deux zones de distincte densité électronique une marginale et l'autre médiane La première possède des fibres et une substance interfibrillaire, la seconde contient seulement des fibres à mailles très lâches qui arrivent jusqu l'épithelium luimême. Par conséquent il y pas d'espace souscupulaire. Dans la zone marginale se situent les otolithes, et dans la zone moyenne, les cils Bien que l'on puisse voir parfois quelques otolithes dans la première nous n'avons jamais trouvé un contact direct entre ces deux structures. Les otolithes ont une longueur comprise entre 6 et 20 microns. Leur corps possèdent deux surfaces de distincte densité électronique: une périférique dense et une autre centrale plus claire. Les extrêmes des otolithes sont formés en grande partie de matière filamenteuse qui possède une orientation perpendiculaire à sa facette.

ZUSAMMENFASSUNG

Man hat die Ultrastruktur der otolithischen Membrane der utriculären Macula des Meerschweinchens untersucht und festgestellt, dass sie wellenartig ist und aus zwei Zonen von verschiedener elektronischer Dichte besteht, einer marginalen und einer medialen. Die erstere besteht aus Fasern und einer zwischen denselben liegenden Grundsubstanz, die zweite enthält ein sehr lockeres Konglomerat von Fasern, die bis ans Epithel heranreichen, das heisst, dass es keinen subcupularen Raum gibt.

In der marginalen Zone befinden sich die Otolithen und in der medialen die Cilien, obwohl man gelegentlich auch in ihr einen Otolith finden kann, aber wir haben nie einen direkten Kontakt zwischen diesen beiden Strukturen vorgefunden.

Die Otolithen haben eine Länge zwischen 6–20 μ Ihr Körper hat zwei Zonen von verschiedener elektronischer Dichte, eine sehr dichte, periphere, und eine zweite zentrale die klarer ist. Die Extremen der Otolithen bestehen zum grossen Teil aus einem faserigen Material das eine Orientierung senkrecht zur Oberfläche aufweist.

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SUBMICROSCOPIC STUDIES OF THE ROUND WINDOW MEMBRANE

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Abstract A study of the ultrastructure of the round window membrane revealed several interesting characteristics of functional significance. The membrane consists of three layers: (1) An external layer faces the tympanic cavity and varies from one to two cells in thickness. It is composed of four types of cells: osmiophilic, osmiophobic, dark granulated and goblet cells. The free surface has microvilli and is coated with an amorphous substance. (2) An internal layer facing the scala tympani, is one cell thick and lacks microvilli, but an occasional kinocilia was observed. The cells have very long thin cytoplasmic processes and extensive endoplasmic reticulum. Secretory granules are present, and it appears that these cells synthesize substance, possibly glycogen. Blood vessels were observed resting on the free surface. (3) A middle layer of connective tissue contains all the normal components and is interspersed with blood vessels and unmyelinated nerve fibers. Both collagen and elastic fibers were found to be quite evenly distributed in radial arrangement throughout this layer

The round and oval windows are the two foramina which lead from the tympanic cavity into the inner ear The oval window which is closed by the stapes, is located at the beginning of the scala vestibuli. The round window on the other hand, leads to the scala tympani and is covered by the round window membrane (also called the secondary tympanic membrane, the fenestra cochlea membrane, and the fenestra rotunda membrane)

The exact function of the round window is still an area of debate. It is generally accepted that the round window membrane acts as a release mechanism for the fluid pressure that is created by the inward movement of the stapes in the oval window during sound stimulation. Sellers (1961) presents evidence suggesting that the round window membrane might function as an alternate pathway for the entrance of sound waves to the cochlea, especially for higher tones. Arslan (1969) in order to create a model for Menière's Syndrome, demonstrated the semipermeability of the round window membrane to perilymph by placing a NaCl crystal on the outer surface of the membrane. He observed an outflow of fluid.

The authors wished to investigate the possibility that the round window membrane might have a secretory and/or absorptive function in addition to having a mechanical function. Though the position, shape and development of the round window have been quite accurately described by Bast & Anson (1952), to our knowledge, this is the first complete study of the ultrastructure of the round window membrane. Accordingly this study was undertaken to examine in detail the ultrastructure of the round window membrane and to correlate morphologic findings with functional possibilities. This will also serve as a basis for a later study of the mode of repair of this membrane following injury

MATERIAL AND METHODS

The guinea pigs (300–400 g) used in this study were decapitated following anesthetization with ether The bullae were rapidly removed

and opened to expose the round window membrane. The preparation was then placed in cold glutaraldehyde solution (5% glutaraldehyde in 0 1 M phosphate buffer pH 7.35) While in this fixative solution, most of the bone surrounding the round window membrane was dissected away and the intact round window membrane with a small ring of bone to which it was attached was isolated.

As a preparation for electron and phase contrast microscopy the specimens were refrigerated for 2 hours in cold, fresh glutaraldehyde solution, followed by a 2 hour post fixation with Palade's fixative (1952) (1% osmium tetroxide buffered with acetate veronal buffer) The specimens were then dehydrated in a series of ethyl alcohol solutions and embedded in epon using Luft's procedure (1961) Using a Sorvall Porter Blum MT 2 ultramicrotome, sections were cut perpendicular to the plane of the membrane and stained with toluidine blue for phase contrast examination. Ultrathin sections were then cut and stained with uranyl acetate and lead hydroxide and examined with Jeolco JEM 7 electron microscope at 80 KV

order to study the distribution of collagen and elastin in the round window membrane, specimens were fixed with 10% formalin, embedded in paraffin, sectioned on a sliding microtome (10 μ – 15 μ thick) stained with Verhoeff's elastic tissue stain, and counterstained in Van Gieson solution (McManus & Mowry 1960)

RESULTS AND DISCUSSION

The round window membrane has a stratified appearance and can be differentiated into three layers (1) an external layer which borders the tympanic cavity and is composed of one to two layers of cells, (2) an internal layer which is bathed in the perilymph of the scala tympani and is one cell in thickness with extremely long cytoplasmic folding, and (3) a middle layer consisting of connective tissue, interspersed with blood vessels. The electron micrograph in Fig. 1 illustrates a cross section of the round window membrane from a central portion. In this region the round window membrane is relatively thin, approximately 8 to 10 μ in thickness and increases to more than 70 μ at the periphery

Tympanic Cavity Layer

The epithelium facing the tympanic cavity in the peripheral portion of the membrane (Fig. 2) is distinguished by two types of cells, osmiophilic (cells with darkly stained cytoplasm) and osmiophobic (cells with lighter cytoplasm) In some portions, this layer is only one cell thick and in other parts two cells thick (varying from 5 to 10 μ) (Fig. 2 A, B) The epithelium is separated from the underlying connective layer by a basal lamina (basement membrane) The cells that make up the epithelium are squamous with their long axis parallel to the membrane's surface. In the portions that are one cell thick, osmiophilic cells line the free surface and are adjacent to one another while the osmiophobic cells lie beneath them (Fig. 2 B). As one proceeds along the membrane from the peripheral portion to the thinner more central portion, the epithelium is made up of only one layer of extremely elongated osmiophilic cells. The epithelium in this region is only 0.5 μ in thickness. In Fig. 2 B the abundance of mitochondria and ribosomes in the extensively folded cytoplasm of the osmiophilic cells is evident. The nuclei of these cells are quite irregular in shape with crypts of cytoplasm.

The osmiophobic cells of the tympanic cavity layer are shown in Fig. 2 A, C. Both mitochondria and rough-surfaced endoplasmic reticulum are present, and the nucleus is less irregular in shape than the nuclei of the surrounding osmiophilic cells. The remnant of a kinocilia should be noted. The cell boundaries between the epithelial cells are not smooth, but rather contains numerous finger-like folds. Occasionally a third type of cell was observed resting on the basal lamina (Fig. 2 A) This cell is osmiophobic and triangularly shaped. It has a smooth, spherical nucleus, rough endoplasmic reticulum, but few mitochondria.

A characteristic common to both the os-

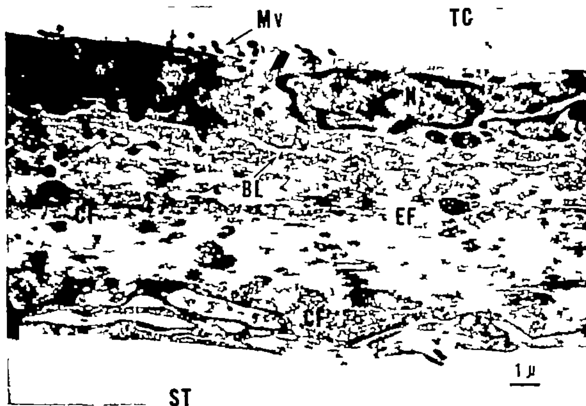


Fig. 1 Electron micrograph of a cross section of the round window membrane showing three layers. The tympanic cavity layer is separated from the middle connective tissue layer by basal lamina (*BL*) *CF* collagen fibers, *EF* elastic fibers *M* microvilli, *N* nucleus, *ST* scala tympani, *TC* tympanic cavity

miophilic and osmiophobic cells on the tympanic cavity surface of the round window membrane is the presence of microvilli, as shown in Fig. 3 These microvilli are quite numerous at the periphery of the membrane, but in the central portion they are usually found only at the cell junctions. In addition, portions of the cell surface were found to be coated with an amorphous substance (Fig. 3 A) A similar finding on the tympanic cavity surface of the tympanic membrane has been reported by Johnson et al. (1968) They suggested that the substance might be a layer of mucus produced by the middle ear

An important finding was the presence of two types of secretory cells of different morphological characteristics: goblet cells and dark granulated cells. The goblet cells are located at the periphery of the round window membrane of the tympanic cavity side and are packed with large, relatively light colored granules (Fig. 4). They are oblong with their long axis parallel to the membrane surface, and they appear to be resting on top of the free surface of the squamous cells. The microvilli of the squamous cells can be seen protruding into the space between the goblet cell and the epithelial surface. It seems that the goblet cells are not a variant form of the squamous cells of the tympanic cavity layer but rather a distinct cell.

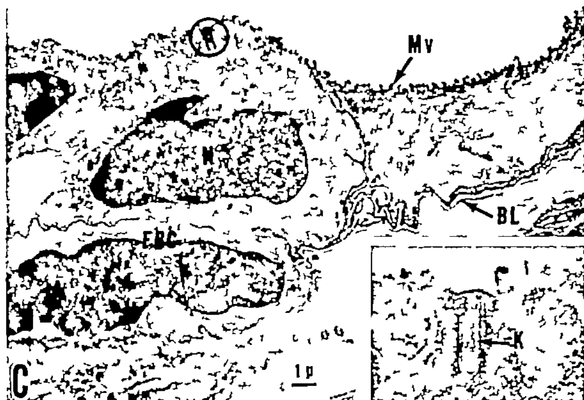
The other type of secretory cell, the dark granulated cell (Fig. 5) is also found most commonly at the periphery of the membrane, though they are occasionally also found more centrally The cell shape and position is like that of the squamous epithelium, and it seems that the dark granulated cell is a modified squamous cell, containing many small, dark, spherical granules. A golgi apparatus is usually present, and the microvilli that line the surface



Fig 2. Osmiophilic and osmiophobic cells of the tympanic cavity layer found near the periphery of the round window membrane. The free surface is lined with microvilli (Mv). In some portions the layer is one cell thick (Fig. A, C) and in other areas two cells thick (Fig. 2 B). Note the triangular cell in Fig. 2 A and the kinocilia remnant (K) (Insert) and fibroblast cell (FBC) in Fig. C. BL, basal lamina, ER, endoplasmic reticulum N nucleus.

of the cell are numerous. Often the cells that lie beneath these secretory cells contain many small vesicles.

The tympanic cavity layer of the round window membrane has been described light microscopically as a continuum of the mucosal lining of the middle ear (Bast & Anson, 1952). Therefore, ultrastructural similarities between the round window membrane and the middle ear mucosa should be expected. Lim et al.



(1967) found the middle ear mucosa covering the tympanic bulla in the guinea pig to be composed basically of squamous epithelial cells with elongated cellular processes and small microvilli. They found some of these cells to contain dark secretory granules. These cells are very similar to the osmiophilic cells and the dark granulated cells that make up the tympanic cavity layer of the round window membrane. Lim et al., however did not report finding any goblet cells in the epithelium lining the bulla, though they did find goblet cells in the epithelium of the Eustachian tube Hussl & Lim (1969) histochemically classified the dark granulated cells of the middle ear mucosa as serous (non-mucous) and the goblet cells as seromucous. It was their opinion that the dark granulated cells probably are distinct cells, not a precursor of the goblet cells.

The striking similarities to the middle ear mucosa suggests that the tympanic cavity epithelium of the round window membrane functions in conjunction with the middle ear mucosa and is producing and secreting substances into the cavity layer

Scala Tympanic Layer

The epithelium on the scala tympani side of the round window membrane which is bathed in perilymph, is made up of one layer of osmiophilic cells (Fig. 6) and is approximately 2 to 4 μ thick. These cells are extremely elongated, as are their nuclei, with the cells long axis parallel to the membrane s surface. The cell membrane and nuclear surfaces are rather smooth. The cytoplasm of these cells is in the form of numerous long thin layers abundant in endoplasmic reticulum. Also, a golgi apparatus is often visible. The insert in Fig. 6 B shows a kinocilia found in an active cell of the scala tympanic layer

At the more peripheral portions of the membrane capillaries are seen resting on the free surface of the scala tympanic layer (Fig. 7) The capillaries appear to be held in place by a very thin cytoplasmic layer (from the cells of





Fig 3 (A) Microvilli (*M*) that line the tympanic cavity surface of the round window membrane. The arrow indicates the amorphous substance which coats the surface. (B) Enlargement of the microvilli (*M*) found at the junction of two cells. Note the irregular border between the cells. *R*, ribosomes; *TC* tympanic cavity

Fig 5 Photomicrograph of a dark granulated cell on the tympanic cavity surface of the round window membrane. Note the vesicles (*V*) in cell of the connective tissue layer below the secretory cell. *BL*, basal lamina, *CF* collagen fibers; *G* golgi apparatus; *M* microvilli *SG* secretory granules.

Fig 4 Portion of goblet cell resting on the surface of the tympanic cavity laye of the round window membrane. Note the separation between the goblet cell and the osmiophilic cells. *BL*, basal lamina, *G* golgi pparatus, *M* microvilli, *TC* tympanic cavity



Fig 6 Photomicrographs of a portion of cell from the scala tympani layer of the round window membrane. The golgi apparatus (*G*) and the thin layering of the cytoplasm should be noted. The insert in Fig. 6 A shows a kinocilia (*K*) with a centriole (*C*) found in one such cell. Inclusion granules (*G*) are visible in Fig. 6 B *CF* collagen fibers *EF* elastic fibers, *N* nucleus, *ST* scala tympani.

the scala tympanic layer) which envelopes the capillary

The authors have observed secretory granules in the outermost portion of the cells. One type of granules appears to be an aggregate of small, extremely dark particles (Fig. 6 B). This form is very similar to the alpha form of glycogen (Fawcett, 1966), commonly found in the cells of the mammalian liver Lead hydroxide which was used in our preparations, is

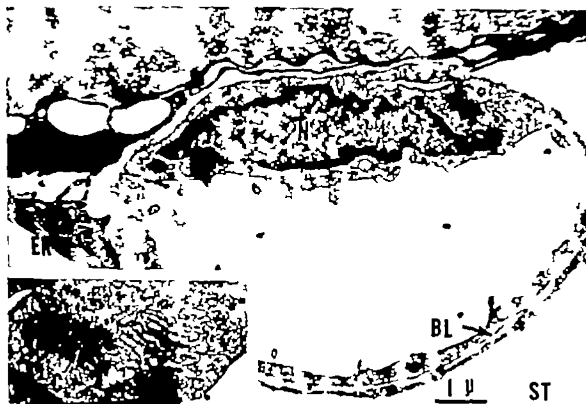


Fig. 7 Photomicrograph of capillary resting on the scala tympani layer of the round window membrane. Note the extremely thin layer of cytoplasm that covers the capillary. Insert is an enlargement view of the thickest portion of the capillary cytoplasm, showing a Golgi apparatus (*G*) and a centriole (*C*). *BL*, basal lamina, *ER*, endoplasmic reticulum. *N* nucleus. *ST* scala tympani.

known to stain glycogen. Granules of another type have also been noted. The authors have not yet identified these granules with certainty but they do correspond closely in size and shape to lysosomes.

The presence of the granules together with the endoplasmic reticulum and golgi strongly indicates synthesis in these cells of a substance which is thus secreted into the perilymph. In addition, the long slender layered cytoplasm and the thinness of the scala tympanic layer itself would greatly facilitate absorption and secretion.

Middle Connective Tissue Layer

Between the outer and inner surfaces of the round window membrane is a wide layer of connective tissue which increases in size as it moves from the center to the periphery of the membrane (Figs. 1 8). It contains many large, elongated fibroblast cells, with long cytoplasmic processes which extend parallel to the round window membrane surface and contain rough endoplasmic reticulum, mitochondria, and occasionally a golgi complex. Often fibroblast cells can be seen in direct contact with the basal lamina of the tympanic cavity layer (Fig. 2 C).

Capillaries are found in the connective tissue, usually very near the basal lamina of the tympanic cavity layer in the peripherial regions. The capillary shown in Fig. 9 A is quite large relative to the surrounding cells and is separated from the osmiophilic cells above it by only a very small area of connective tissue, but it is not in direct contact with the cells. This is in contrast to the capillaries of the scala tympanic side of the round window mem-



Fig. 8. Tympanic cavity side of the round window membrane is shown. A basal lamina (*BL*) clearly divides the cytoplasm of the osmiophilic cells, from the connective tissue with a nucleus (*N*) of fibroblast cell, collagen fibers (*CF*), and elastic fibers (*EF*). Note the amorphous coating with microvilli (*Mv*) of the osmiophilic cell. *Pv* pinocytotic vesicle. *TC* tympanic cavity.



Fig 9 (A) Photomicrograph showing the relationship between a capillary lying in the connective tissue layer to the osmiophilic cells of the tympanic cavity layer of the round window membrane. The basal laminae, surrounding the capillary and underlying the osmiophilic cells are clearly visible. (B) An area of connective tissue surrounding a capillary. Unmyelinated nerve fibers (*NF*) are located near nucleus (*N*) of the capillary wall. *E*, erythrocyte; *ER*, endoplasmic reticulum, *M* microvilli; *N* nucleus, *LC* lumen of capillary. *TC* tympanic cavity



brane which lie entirely outside the connective layer on the free surface of the scala tympanic

" Unmyelinated nerve fibers have observed in close proximity to the capill- as shown in Fig. 9 B

The distribution of collagen fibers and elastic fibers can be seen clearly in Figs. 1 8 The elastic fibers appear to be uniformly distributed throughout the connective tissue layer for the entire length of the round window membrane This is contrary to the histochemical findings of Hardy (1963) which indicated that elastic fibers were found only in the thin central portion of the round window membrane mostly under the epithelia. Collagen fibers are arranged radially and are quite evenly distributed throughout the connective tissue layer except at the periphery of the round window membrane where they tend to be more greatly concentrated on the tympanic cavity side. These findings were confirmed by the authors histochemically using Verhoeff's elastic tissue stain combined with Van Gieson's solution. The radial arrangement of collagen fibers, together with the presence of elastic fibers, gives the round window membrane its necessary strength and flexibility

In conclusion, the epithelia of the round window membrane bordering the tympanic cavity and the scala tympani both appear to be metabolically active. The authors, based upon electron microscopic observations, suggest the round window membrane may be bifunctional, not merely a membrane to prohibit the escape of fluid and dissipate fluid movement, but may also be capable of secretion and/or absorption.

ACKNOWLEDGMENT

The authors would like to express their gratitude to Dr E. N Myers for his valuable constructive suggestions.

ZUSAMMENFASSUNG

Bei der Untersuchung der Membrane des Runden Fensters wurden verschiedene Eigenschaften von funktioneller Wichtigkeit gefunden. Die Membrane

besteht aus drei Schichten. 1 Eine äussere Schicht auf der Seite der Paukenhöhle. Sie variiert in der Dicke zwischen ein und zwei Zellenlagen. Sie besteht aus vier Zellarten, osmophile osmophobe Zellen mit dunklen Körnern und Becherzellen. Die freie Oberfläche ist mit Mikrovilli besetzt und ist mit einer amorphen Substanz bedeckt. 2. Eine innere Schicht, auf der Seite der Scala tympani. Sie ist eine Zellenlage dick und weist keine Mikrovilli auf, hin und wieder wurde eine Kinozilia gefunden. Diese Zellen sind sehr lang und dünn und besitzen viel endoplasmatisches Retikulum. Sekretionskorner wurden auch beobachtet. Es scheint, dass diese Zellen eine Substanz synthesieren, möglicherweise Glykogen. Blutgefässe befinden sich ruhend auf der freien Oberfläche. 3 Eine mittlere Schicht von Bindegewebe enthält alle normalen Organellen und ist von Blutgefässen durchzogen. Kollagen und elastische Fibern sind gleichmässig in radialer Anordnung durch die ganze Schicht verteilt.

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QUANTITATIVE ANALYSIS OF ACID MUCOPOLYSACCHARIDES IN THE NORMAL AND KANAMYCIN INTOXICATED COCHLEA

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Abstract Quantitative analysis of acid mucopolysaccharides in the kanamycin intoxicated guinea pig cochlea was performed using the modified turbidimetric method based on the formation of insoluble complexes between acid mucopolysaccharides and cetyltrimethylammonium bromide, in combination with enzymic analysis. A decreased quantity of acid mucopolysaccharides i the stria vascularis and the spiral ligament was observed in comparison with the normal. The factors involved in the decrease of acid mucopolysaccharides and its effect on the inner ear function were discussed.

1953 Belanger reported finding several sul- compounds in the tectorial membrane. Since then there has been a great interest shown in the role of mucopolysaccharides (MPS) in the hearing process (Dohlman, 1960 Vilstrup & Jensen, 1961) The finding of deafness as a frequent symptom in mucopolysaccharidoses has further stimulated interest in their identification and measurement (Iurato, 1960 Schubert & Hamerman, 1968 Saito & Daly 1970) The present study is designed to compare the quantity of MPS in normal cochleae and in the cochlea of kanamycin intoxicated guinea pigs.

METHOD

The difficulties of quantitatively measuring the amount of acid mucopolysaccharides (AMPS) in the tectorial membrane and the basal membrane were due to the small amount of materials available for the assay and the minute amount of AMPS contained in these tissues. In order to get sufficient amount of tissue the stria vascularis with the spiral ligament were selected for the first quantitative comparison. To make the assay in other parts of the cochlea, tissues from several animals were collected and their mean values were determined.

Preparation of Animals

Eighty colored guinea pigs with a normal pinna reflex, weighing between 250 and 350 gm were used. These were divided into two groups.

(*a*) Twenty were used for the quantitative comparison of total AMPS in the stria vascularis with the spiral ligament, between normal and kanamycin intoxication. Eight were used for control, twelve for kanamycin.

(*b*) Sixty were used for the detailed analysis of AMPS in kanamycin intoxication.

Kanamycin intoxication was produced by subcutaneous injection of 400 mg of kanamycin sulfate (potency 770 μg/mg supplied by Bristol Laboratories, Syracuse, N Y) per kg of body weight daily (except weekends) for 10 days. During the course of injection, 4 out of 72 animals died. On the day following the last injection, animals were sacrificed by cervical dislocation.

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Preparation of Tissues

The temporal bones were kept in neutral 10% formalin at room temperature from 1 to 4 days. Dissection was made in absolute alcohol under a stereoscopic microscope without staining. From group (*a*) only the stria vascularis with the spiral ligament was collected in acetone. They were divided into five small groups for five measurements of both control animals and those receiving kanamycin. The kanamycin membranous cochlea of group (*b*) were divided into three parts, (1) the stria vascularis with the spiral ligament, (2) the tectorial membrane, and (3) the basal membrane with the organ of Corti and limbus spiralis. They were collected and stored in acetone The stria vascularis with the spiral ligament was divided into two groups.

Measurement of Dry Weight

A quartz helical balance (Microchemical Specialities Company) whose sensitivity was 12.7 μg per 1 mm deviation was used. After drying in a desiccator for over 3 days, dry weight of the tissues was measured. The mean value of each dried tissue per cochlea of *group (b)* was measured to compare with that of the normal.

Extraction of Acid Mucopolysaccharides (AMPS) from Tissue

The procedure was essentially the same as that described previously (Saito & Daly 1970) The precipitate, containing pure AMPS throughout the procedure, was dissolved in acetate buffer (pH 6.0) from 0.5 to 2.0 ml according to the amount. This solution, centrifuged if necessary was used for turbidimetric measurement of AMPS

Measurement of Acid Mucopolysaccharides

The method was the same as that reported previously (Saito & Daly 1970)

Maximum sensitivity was 1 μg/ml or 0.5 μg per 0.5 ml. Recovery tests showed 95 4 ± 2.0% (mean ± standard deviation of 10 determinations) recovered with a fine pipette and a refined technique. Blank tests through the whole procedure always showed under 0 05 O.D However the blank tube and the recovery test tube containing 100 μg of chondroitin sulfate A (ChS-A) throughout the procedure were always prepared to check the technical failure and to correct the blank.

Kanamycin sulfate showed no turbidity at a concentration from 1% to 0 01% The only important interfering substance was deoxyribonucleic acid (DNA), which showed about 92% of turbidity compared with ChS-A as 100%

Enzymic Analysis of Acid Mucopolysaccharides

Enzymes employed in this experiment were deoxyribonuclease (DNase) and testicular hyaluronidase (HAase) The detailed procedure was the same employed previously (Saito & Daly 1970) The total amount of genuine AMPS, HAase digestive AMPS and HAase-resistant AMPS was obtained from enzymic analysis.

RESULTS

1 *Dry weight of the kanamycin-intoxicated membranous cochlea*

The mean value of dry weight of the kanamycin-intoxicated membranous cochlea of group (*b*) was obtained to compare with that *of normal* (Saito & Daly 1970). The results showed that the tectorial membrane per cochlea weighed 4 4 μg, the basal membrane with the organ of Corti and limbus spiralis 59 4 μg and the stria vascularis with the spiral ligament 230 0 μg. The total D W per one kanamycin intoxicated membranous cochlea was 293 8 μg, while that of normal was 293 9 Reissner's membrane was so light that it was considered to be included within the range of error (Saito & Daly 1970) No change in weight was observed at this dosage (400 mg/kg of body weight for 10 days). The details are shown in Table I.

2. Quantitative analysis of acid mucopolysaccharides in kanamycin-intoxicated membranous cochlea and its comparison with normal

The total amount of AMPS in the stria vascularis with the spiral ligament of normal and kanamycin-intoxicated from group (*a*) was obtained after decomposition of DNA with DNase. The normal contained 0.51 ± 0 08 % (mean ± standard deviation) AMPS per dry weight, while kanamycin intoxication contained 0 31 ± 0 04 % (Table II)

A *t* test showed 5 03 with 8 degrees of freedom which is almost at the 0 1 % level of significance Thus it becomes evident that the difference is statistically significant therefore, the decrease of AMPS in the stria vascularis and spiral ligament due to kanamycin intoxication is highly significant.

The analysis of kanamycin intoxication from group (*b*) showed that the tectorial membrane contained 0 15 % AMPS per dry weight, the basal membrane with the organ of Corti and limbus spiralis 0 43 % and the stria vascularis with the spiral ligament 0.39 % All of the percentages are the mean of 116 cochleae Further enzymic analysis of the tectorial membne was not possible due to the small amount. ever the decomposition of DNA was not ssary since it had no cell component arato 1962) Details are shown in Table III.

Table I. *Comparison of dry weight of the membranous cochlea of normal and kanamycin-intoxicated guinea pig (body weight from 250 to 350 gm)*

Part of cochlea	Condition	Cochlea (*n*)	D W Cochlea (μg)
Tectorial membrane	Normal	450	4 6
	Kanamycin	116	4 4
Basal membrane with organ of Corti and limbus spiralis	Normal	418	60.3
	Kanamycin	116	59 4
Stria vascularis with spiral ligament	Normal	407	230 0
	Kanamycin	116	230.0
Total membranous cochlea	Normal	450	293.9
	Kanamycin	116	293 8

Table II. *Quantitative comparison of acid mucopolysaccharides in the stria vascularis with the spiral ligament between normal (N) and kanamycin intoxication (KM)*

Measurement no.	D W (μg)	AMPS extracted (μg)	%
N 1	753.1	4.5	0.60
N 2	769.6	3.5	0 46
N 3	1 002.0	5.5	0.55
N 4	753 1	3.0	0 40
N 5	755 7	4 1	0.54
Mean ± S.D			0.51 ± 0 08
KM 1	972.8	3.0	0.31
KM 2	961 4	2.5	0.26
KM 3	994 4	3.0	0.30
KM 4	1 022.4	3.8	0.37
KM 5	972.8	3 1	0.32
Mean ± S.D			0.31 ± 0.04

In the normal, the tectorial membrane contained 0 11 % AMPS per dry weight (mean of 480 cochleae), the basal membrane with the organ of Corti and limbus spiralis 0 49 % (mean of 356), and the stria vascularis with the spiral ligament 0 61 % (mean of 291) (Saito & Daly 1970)

Fig 1 shows the comparison of the mean content of AMPS in the membranous cochlea between normal and kanamycin intoxication of group (*b*)

The change of total AMPS in the stria vascularis with the spiral ligament is highly significant as described above. Most of the decreased AMPS in this part seems to be HAase digestive AMPS such as hyaluronic acid, ChS-A or ChS-C. It is also suggested that the changes of AMPS in the other parts are not as large as the stria vascularis and the spiral ligament

DISCUSSION

It has been reported by Higginbotham (1958) and Mora et al. (1959) that AMPS in connective tissue or anionic derivatives of synthetic polyglucoses reduced toxicity of cationic macromolecules, such as polymyxin B streptomycin

Table III *Analysis of each part of the kanamycin intoxicated membranous cochlea*

Tissues	Tectorial membrane	Basal membrane with organ of Corti and limbus spiralis	Stria vascularis with spiral ligament	
Total D W (µg)	509.3	6 892.3	11 579.9	15 096.5
CTAB-complex (µg)	0.8	33 6	46 4	73 6
AMPS (µg) (/D W)	0.8 (0.15)	29.6 (0.43)	40.8 (0.35)	63 2 (0.42)
HAase digest. (/AMPS)	—	20 0 (68)	20 8 (51)	32.8 (52)
HAase resist. (/AMPS)	—	9 6 (3)	20.0 (49)	30.4 (45)
DNA (µg) (/D W)	—	4 0 (0 06)	5.6 (0.05)	10 4 (0 07)

or kanamycin. The formation of drug-mucopolysaccharide and its digestion by fibroblasts were suggested (Higginbotham 1958)

On the other hand, the reaction between 0 01 % kanamycin solution and 0 01 % ChS-A solution or the solution extracted from the stria vascularis with the spiral ligament in vitro showed no effect upon the complex formation with CTAB-Li reagent up to 24 hours incubation. This suggested that kanamycin did not directly decompose AMPS, and that AMPS reacted more strongly with CTAB than with kanamycin.

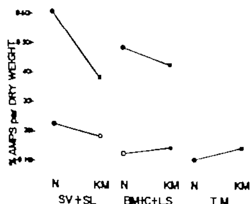


Fig 1 Comparison of the mean content of acid mucopolysaccharides in the membranous cochlea between normal and kanamycin intoxication. *SV* stria vascularis; *SL*, spiral ligament; *BM* basal membrane; *C* organ of Corti, *TM* tectorial membrane. ● ○ indicate normal (*N*); ■ □, kanamycin intoxication (*KM*); infilled symbols, total AMPS open symbols, HAase-resistant AMPS infilled symbols minus open, HAase digestive AMPS.

Saito (1967) has studied the enzymic synthesis of ChS in the guinea pig cochlea using a radioisotope. According to that report, the activation of sulfate, that is, PAPS (3 -phosphoadenosine 5 -phosphosulfate) synthesizing enzymic activity was decreased in kanamycin intoxication It has been reported by Haba & Holtzer (1965) that puromycin also inhibits synthesis of ChS coupled with that of protein.

Therefore, the decrease of AMPS in the present study was considered to be due to two factors. First is the formation of drug-mucopolysaccharide complexes and its digestion by fibroblasts. Second is an inhibition of synthetic process of AMPS coupled with that of protein, since both kanamycin and puromycin are inhibitors of protein synthesis.

Milsebeck & Schätzle (1964) have histochemically investigated the behavior of the guinea pig cochlear AMPS after dihydrostreptomycin and tetracyclin derivative poisoning. A decrease of HAase digestive AMPS, especially in the limbus spiralis, after dihydrostreptomycin poison was reported, while a tetracyclin poisoning slightly lowered the reaction only in the limbus. The decrease of HAase digestive AMPS

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PERMEABILITY OF REISSNER'S MEMBRANE IN THE ISOLATED EAR OF THE GUINEA PIG

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(Received September 3 1970)

Abstract The purpose of this work was to provide more information concerning the permeability f Reissner' membrane. Examinations were carried out using chemical compounds labelled with radioisotopes. The following molecules wer used: NaCl, KCl, NaJ $NaHCO_3$, Na_2HPO Na_2SO albumin and colloidal gold. Experiments were carried out on 77 isolated labyrinths of the guinea pig. Isotope solution was introduced into the space of the scala vestibuli through the oval window opening. The activity of the compound in the scala media, scala vestibuli and scala tympani was determined after 4 minutes. Estimation of permeability was based on the relative activity of the compound on both sides of Reissner's membrane. It was found that small inorganic compounds penetrate Reissner's membrane but albumin and colloidal gold did not. Penetration of the small inorganic compounds demonstrated remarkable variations. Potassium and sodium had the greatest ability to penetrate, and iodine and bicarbonate the least. Orthophosphoran and sulphate showed slight penetration. The results demonstrate some similarity in permeability of Reissner's membrane and capillary vessels. Exchange of some molecules between the perilymph of the scala vestibuli and the endolymph of the cochlear duct confirms the theory of "the radial flow" of the labyrinth fluids, yet not proving it entirely

Current scientific methods have not as yet explained completely the function of Reissner's membrane. The purpose of this work is to examine and determine the permeability of this membrane for some particles present in labyrinthine fluids.

Many investigators thought the membrane to be more or less permeable as far as the function is concerned. Rudinger Bast, Sakren, Meyer Mygind citing Rauch (1964), Altmann & Waltner (1947) Lawrence et al. (1961) Naftalin & Harrison (1958) Meyer zum Gottesberge et al. (1965) Rauch et al. (1963) Rauch (1966) Pratma (1969 *a*, *b*) However the results of electrophysiological examinations upheld the opinion that Reissner's membrane is not permeable Davis (1957 1958) Tasaki & Spyropoulos (1959) Vosteen (1961) and others consider the membrane to be completely impermeable

Respirometric investigations carried out by Chou (1963) showed remarkable consumption of oxygen by this tissue. Kawata & Hayata (1966) found phosphorylases in it, which most probable is not found in the vascular stripe.

Autoradiographic examination of Reissner's membrane showed energetic incorporation of leucine (Plester 1960; Meyer zum Gottesberge & Plester 1961) Investigations of Rauch & Köstlin (1962) Rauch et al (1963) Rauch (1966) showed that there was an exchange of sodium and potassium between the scala vestibuli and the scala media They proved that the ions penetrate Reissner's membrane after interdiction of the blood circulation. Wandering of sodium and potassium ions does not prove that the membrane is involved in the process of component exchange between the perilymph of the scala vestibuli and endolymph.

Investigations of other molecules penetrating of Reissner's membrane could to



Fig 1 Introduction of isotope solution into the scala vestibuli. 40. *O OW* oval window *S* stapes head, *K*, capillary filled with isotope solution.

tent provide more information concerning the function and biological properties of this part of membranous labyrinth.

MATERIAL AND METHODS

Seventy-seven male and female white guinea pigs, weighing 150–250 g were used in the ex riments. The animals were divided into eight ps. In each group the animals were given substance at equal time intervals (always 4 in) to examine its penetration of the mem brane. The behaviour of the following eight compounds and molecules was investigated. KCl (K-42) NaCl (Na-24) $NaHCO_3$ (C 14), Na_2HPO (P 32) Na_2SO_4 (S-35) NaJ (J 131) albumin (J 131) colloidal gold (Au 198)

The animals were decapitated without premedication. The bone case together with the temporal bone were then separated. The revealed labyrinth was placed in the micromanipulator During the experiment the temperature of the labyrinth was maintained at 37 C. The stapes was moved aside to make the scala vestibuli accessible. This step was made with help of stereoscopic microscope PZO MST

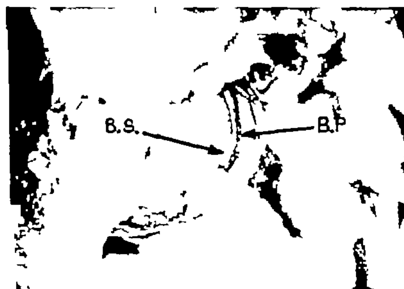


Fig 2 The basilar membrane after removing the bone wall of the basal turn covering the scala tympani. 40. *B.P* basilar membrane *B.S* spiralis ossea.



Fig. 3 Taking samples of endolymph ×10. *B.P* basilar membrane *K* capillary *M.P* puncture of the basilar membrane.

130. A calibrated capillary filled with labelled solution of the compound was introduced into the gap between the stapes edge and periosteum restricting the oval opening, by means of the micromanipulator (Fig. 1)

The volume of the isotope thus given was a constant 1 μl. The time from the moment of introducing the isotope to the moment of taking samples was measured and in the meantime observations concerning permeability were made. A thin steel needle was used to remove the bone of the basal turn covering the scala tympani. Then the whole perilymph was removed and the basilar membrane carefully dried (Fig. 2)

About 10 sec later a sample of endolymph was taken (Fig. 3) Endolymph was sampled as described by Aldred et al. (1940) Several seconds later a sample perilymph in the scala vestibuli was aspirated through the oval opening (Fig. 4) Quantities of the endolymph obtained varied, i.e., 0 05–0 4 μl. Reissner's membrane was carefully controlled after taking samples to ascertain possible damage. The activity was measured by Geiger-Müller counter with electronic recast type LL 1

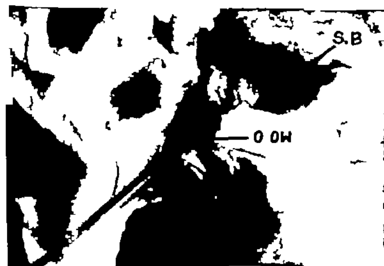


Fig. 4 Taking samples of perilymph from the scala vestibuli. *S.B* scala vestibuli, *O.OW* oval window

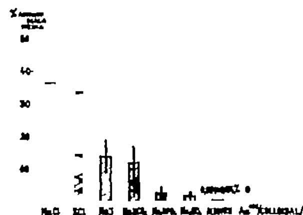


Fig 5 Graphical presentation of the endolymph relative activity when examining penetration of particular compounds.

ity was found in the endolymph containing sulphur S-35 (0 7 ± 0 17 %) When examining the permeability of albumin labelled J 131 the vestigial activity of the element was determined in the endolymph (0 05 ± 0 13 %) Colloidal gold was not found in the endolymph of any of the ears

The small number of measurements in particular groups does not allow statistical estimation of differences in contents of particular compounds in the endolymph. The perilymph activity of the scala tympani had different values in each group The differences did not show any noticeable regularity

RESULTS

Estimation of permeability was based on the relative activity of compound on the both sides of Reissner s membrane. Assuming perilymph activity in the scala vestibuli to be 100 % one could estimate endolymph activity in the scala media as a percent age magnitude thereof.

Quantities of potassium K-42 found in the ndolymph were greatest (36 6 ± 20 8 %) ntities of sodium were nearly the same 5 ± 20.2 %) Significant standard devia s did not allow a determining of the differences in the contents of the endolymph tracers. The quantity of iodine in the endolymph was three times smaller than the quantity of sodium and potassium, J 131 (12.4 ± 3 46 %) Bicarbonate C 14 had a similar activity in the endolymph to iodine (11 6 ± 4 6 %) The quantity of phosphorus was much smaller (1 65 ± 1 95 %) Samples in which phosphorus was estimated showed remarkable differences still less activ-

DISCUSSION

The purpose of this study was to examine the behaviour of chemical compounds present in the labyrinthine fluids. Relative concentrations on both sides of Reissner's membrane with identical time intervals and a comparison of the permeability of different substances through the examined membrane were the basis for the estimation of permeability

The investigation was carried out on isolated ears so as to exclude transport of molecules by the blood stream. Migration of molecules between the scala vestibuli and the cochlear duct should be considered as the result of transport mainly via Reissner's membrane The disadvantage of isolating the labyrinth is the difficulty in estimating its function in vivo

To draw conclusions based on the results obtained from the isolated organ is risky It is generally known that isolation of an organ from the organism evokes many different

Table I *Numerical presentation of the endolymph relative activity when examining the penetration of particular compounds*

Investigation particle	KCl	NaCl	NaJ	Na HCO$_3$	Na$_2$HPO	Na$_2$SO	Albumin	Au (colloidal)
Activity I scala Media, %	36.6 ± 20.8	32.5 ± 20.2	12.4 ± 3.46	11.6 ± 4.6	1 65 ± 1 95	0.7 ± 0.17	0.051 ± 0.13	—

changes from both physiological and biochemical points of view. Yet not all life functions come to a standstill during the few minutes after killing an animal.

According to Winnikow & Titowa (1961) the labyrinth maintains most of its life activities even an hour after decapitation. Menzio et al. (1965) have demonstrated a remarkable tolerance of the inner ear to changes in temperature with respect to the generation of microphonic potentials. Other authors think that life functions of the ear die simultaneously with the decrease in body temperature (Gulick & Cutt, 1962). Wever et al. (1941) observed a distinct decrease of microphonic responses after the animal's death, which nevertheless persisted for several hours. The author suggests that the function of the hair cells is independent of the oxygen supply (Wever et al., 1949). Gulick's opinion (1958) is more cautious and he does not correlate the changes in function of the hair cells with moderate insufficiency of oxygen.

On the other hand Perlman et al. (1959) think that the ear is strictly dependent on oxygen supply. It is therefore difficult to form an adamant opinion concerning the changes in life functions of the isolated labyrinth when different authors have such different opinions.

Hitherto, few papers have been published dealing with this problem (Rauch et al. 1963, Ilberg & Imamura, 1966, Prażma, 1969 *a,b*).

Most often the problem of labyrinthine membrane permeability has been considered theoretically in papers concerning the physiology of labyrinthine fluid (Altmann & Waltner 1947, Choo & Tabowitz, 1964, 1965, Citron & Exley 1957, Citron et al., 1956, Kirikae et al., 1961, Lawrence et al., 1961, Naftalin & Harrison, 1958, Rüedi, 1951, Waltner 1948). The problem is treated and explained differently by different authors.

Some authors think that there is a strict connection between ear function and the permeability processes taking place in membrane structures of the labyrinth (Misrahy et al., 1960). Others, such as Lempert et al. (1954) think that the labyrinthine wall has no permeability at all. Recent studies of Prażma (1969*a*, *b*) have proved that there is both active and passive transport of univalent ions through Reissner's membrane. Comparison of the results of this work with the earlier results of Rauch & Köstlin (1962), Rauch (1964), Rauch (1966) shows similarity in sodium and potassium penetration of Reissner's membrane. But it was not possible to determine the quantitative differences in sodium and potassium exchange. The distinct exchange of iodine found between the perilymph of the scala vestibuli and the cochlea endolymph contradicts Misrahy et al. (1960). Inorganic phosphorus and bicarbonate compounds are components of buffer combinations in organisms. An attempt to estimate their ability to penetrate Reissner's membrane is a further means of determining the membrane's properties. Results concerning bicarbonate transport are insufficient to draw really important conclusions. Undoubtedly the process of oxidation of glucose produces carbon dioxide, which must be expelled somehow. There is no evidence to deny that Reissner's membrane is involved in bicarbonate exchange since carbonic acid anhydrase is found in perilymph (Gieldanowski & Prastowski, 1965). But the same level of bicarbonate in endolymph and perilymph suggests that the exchange is somehow controlled. Bialkali dibasic orthophosphorus sodium showed little ability to penetrate. It also penetrates slowly across other barriers (Krochmalska et al., 1966). It may be suggested then that its penetration of Reissner's membrane and capillary walls is similar. Experiments with inorganic sulphate showed its low penetration of Reissner's membrane. This should be accepted as one of the general processes taking place in Reissner's membrane. Belanger's (1953) investigations showed that sulphate takes part in metabolic processes of the membranous labyrinth. Ormerod (1960) found that sulphate penetrates from blood into the labyrinth fluids.

Measurements carried out when labelled albumin was given, provided interesting informa-

tion concerning the penetration of Reissner's membrane. Analysis of the results suggests that such penetration does not occur Slight activity in some samples was, rather the result of free iodine which is always present in the labelled albumin. In one case the value of 0 1% is difficult to explain. Reissner's membrane seems to act like the capillary walls of the labyrinth. Vessels of the inner ear even in pathological condition, e.g. histamine shock, do not show albumin penetration (Baldassini & Bonaccorsi 1964) It might be thought that other proteins with greater molecular weight than albumin do not penetrate Reissner's membrane Experiments with colloidal gold showed that this is not so

The activity of the perilymph of the scala tympani showed entirely different values. However it may be explained by the direct introduction of this compound through the helicotrema. Transport via this pathway showed remarkable individual differences.

Generally it may be said that small molecules of inorganic compounds penetrate Reissner's membrane whereas larger molecules, uch as albumin, do not penetrate. This statent agrees with Coassolo (1956) who examthe permeability of the membranous semicircular walls and observed inorganic small molecules.

The character of the presented permeability of Reissner's membrane confirms the theory of "the radial flow" of the labyrinthine fluids. But to prove the theory it is necessary to carry out further investigations on the permeability of Reissner's membrane and other walls of the membranous labyrinth in vivo.

CONCLUSIONS

1 Small inorganic compounds penetrate Reissner's membrane of the isolated inner ear
2. The penetration demonstrates remarkable variations.
3 Albumin and colloidal gold do not penetrate across the membrane

ZUSAMMENFASSUNG

Das Ziel der Arbeit war das Sammeln neuer Erfahrungen über die Permeabilität der Reissner Membran. In den Untersuchungen wurden markierte Radioisotopen angewandt. Es wurden folgende Verbindungen untersucht. NaCl, KCl, NaJ NaHCO₃ N HPO Na SO Albumin und kolloidales Gold. Die Versuche wurden an 77 isolierten Meerschweinchenlabyrinthen vorgenommen. Die Isotoplösung wurde durch das ovale Fensterchen in die Scala vestibuli eingeführt, und 4 min danach wurde die Aktivität dieser Verbindung im Schneckengang und in der Scala vestibuli bestimmt. Die Permeabilität der Reissner Membran wurde anhand der relativen Verteilung der Aktivität der untersuchten Verbindung zu beiden Seiten der Membran beurteilt. Es wurde eine Permeabilität der Reissner Membran gegenüber der mikropartikularen, anorganischen Verbindungen und ein Fehlen der Permeabilität gegenüber des Albumins und der kolloiden Goldpartikelchen festgestellt. Die Permeabilität der mikropartikularen Verbindungen durch die Membran war verschieden. Die höchste Penetranz zeigten K und Na, die geringste J und das Bicarbonat. Im Falle des Orthophosphates und Sulphates wurde eine nur geringe Permeabilität beobachtet. Die erzielten Ergebnisse weisen bestimmte Analogien zwischen de Permeabilität der Reissner Membran und der Kapillarwände auf. Das Bestehen eines Austausches zwischen der Perilymphe der Scala vestibuli und der Endolymphe des Schneckenganges erlaubt zwar die Annahme der Hypothese einer radialen Durchströmung der Labyrinthflüssigkeit, gibt aber dafür doch keinen Beweis.

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UPTAKE OF RADIOACTIVE STRONTIUM (SR-85) IN AN INFLAMED MASTOID PROCESS

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(Received August 21 1970)

Abstract The activity of inflammation of the mastoid process was measured in 23 patients on the principle that radioactive strontium is metabolised in the same way as calcium in inflammatory processes in the bones. The control material comprised 16 persons with no inflammatory ear disease. The gamma radia tion emitted by Sr-85 in each mastoid process was measured separately by external counting. The results for the diseased and healthy mastoid processes were compared. The difference between the patients with mastoiditis and the controls was statistically significant. The Sr-85 uptake was greatest in the patients with an active inflammation in the mastoid air ll system. The uptake was weaker in the cases with small sclerotic mastoid process or sequelae of in ation. Isotope study is a new method of study the mineral metabolism of the mastoid process, but the procedure should be refined before it can be applied in clinical use.

No reliable quantitative method has been found so far for evaluation of the inflammatory ac tivity in a mastoid process. The patients subjective symptoms, the sedimentation rate and leucocytosis are the criteria usually employed. X-ray examination reveals the inflammatory changes in the mastoid air cell system, but it is difficult to form a clear idea of the activity of the inflammation in this way Nor is it feasible to take samples as the inflammation is intraosseous.

Bauer & Scoccianti (1961) observed the uptake of radioactive strontium in the region of the inflammatory bony process in the spine. Sr-85 was used by Kettunen & Rekonen (1968) to follow the activity of tuberculosis of the bone. Their method was based on the similarity between the metabolism of strontium and calcium in the bones. A local inflammatory process in the bone of the mastoid process causes local acceleration of the calcium metabolism which can be measured externally with Sr-85 as tracer Hence it seems that the method can be applied also to studying the activity of inflammation of the mastoid process.

The purpose of our study was to ascertain (I)—whether the Sr-85 uptake increases in inflammation of the mastoid process (II)—how the intensity of the uptake depends of the nature of the inflammatory process (III)—whether the method is of practical significance for the diagnosis of the inflammatory mastoid process.

MATERIAL AND METHODS

The material consisted of 23 patients with unilateral otitis. Two patients had acute mastoiditis, the others had chronic otitis media or its sequela. One patient also had chronic granulat ing inflammation in the auditory meatus and chronic myringitis. Most of the patients with chronic otitis media or its after-condition were operated on after isotope study At operation, the conditions in the mastoid process were verified grossly and microscopically from tissue specimens.

The control material comprised 16 persons with no ear disease An Sr-85 study was per

Table I *Group I Sr-85 uptake 0–5 %*

Patient	Age	Sex	Sr-85 ( )	Clinical condition, X-ray examination	Surgical findings
1. V. J.	12	♂	0	Chronic serous otitis. Normal mastoid air cell system.	Silastic drain tube.
2. M. K.	15	♂	0	Chronic discharging ear with central posterior pars tensa perforation. Normal mastoid air cell system.	Simple mastoidectomy and myringoplasty. The mucosa in the mastoid air cells was normal. Some thickened mucosa and granulation tissue in the attic.
3. E. M.	53	♀	2	Chronic discharging ear with large marginal perforation. The mastoid process very small and sclerotic.	Radical mastoidectomy: The small mastoid process almost completely sclerosed. The few remaining air cells contained thickened mucosa. Cholesteatoma extending into the antrum.
4. R. P.	22	♂	3	Dry ear with marginal posterior pars tensa perforation, and tympanal cholesteatoma. The large mastoid air cell system partly shattered.	Radical mastoidectomy: The large mastoid process contained air cells lined with normal mucosa in a limited area the cells contained granulation tissue or exudate.
5. E. K.	46	♀	4	Chronic ear with scanty mucoid discharge. Large central pars tensa perforation. The middle-sized mastoid process nearly normal.	Radical mastoidectomy: The mastoid process contained air cells with normal mucosa. A few cells contained granulation tissue. Tympanal mucous membrane swollen.
6. P. K.	60	♂	4	Dry ear with an epitympanic perforation. The mastoid process small and shattered.	Radical mastoidectomy: An attic cholesteatoma. The small mastoid process poorly pneumatized, the cells contained granulation tissue.
7. M. S.	38	♀	4	Chronic discharging ear with large central pars tensa perforation. The mastoid process small and sclerotic.	Radical mastoidectomy: Tympanal cholesteatoma. The mastoid process very small, sclerotic; the remaining few cells contained exudate under pressure.
8. H. P.	28	♂	4	Chronic discharging ear with large central pars tensa perforation. The mastoid process of middle size and partly shattered.	Antrotomy and myringoplasty: The antrum and mastoid air cells were lined with normal mucosa.

formed on these patients for the diagnosis of another osseous disease.

The isotope used in the study was Sr-85 as strontium chloride. The dose was 25 μCi, administered by intravenous injection. The half life of the Sr-85 is 65 days and its radiation is gamma radiation of 511 keV energy.

In the majority of the cases the isotope measurements were made within 24 hours of injecting the tracer. For some patients the meas-

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The control material comprised 16 persons with no ear disease. An Sr 85 study was per

Table III *Group III Sr–85 uptake 11–20 %*

Patient	Age	Sex	Sr–85 ( )	Clinical condition, X-ray examination	Surgical findings
1 H P	37	♀	11	Chronic discharging ear with central pars tensa perforation. The mastoid process of middle-size and shattered	Radical mastoidectomy. The mastoid cell system of middle size, poorly pneumatized. The cells filled with granulation tissue.
2. K. N.	45	♂	11	Chronic discharging ear with epitympanic perforation. Mastoid process small and shattered.	Radical mastoidectomy. The mastoid cell system of rather small size, the cells filled with exudate and/or granulation tissue.
3 M S.	37	♂	12	Chronic discharging ear with large, central pars tensa perforation. The middle-sized mastoid process shattered	Discharge ceased under conservative treatment. The patient refused an operation.
4 R. T	22		16	Chronic discharging ear with marginal pars tensa perforation and cholesteatoma extending int the tympanum. The large mastoid air cell system partly shattered.	Radical mastoidectomy. The cells in the large mastoid process partly pneumatized, partly filled with retained or organized exudate.
5 V L.	37	♂	16	Chronic discharging ear with large pars tensa perforation and an epitympanic perforation with a cholesteatoma. The mastoid process of middle size, cloudy and sclerotic.	Radical mastoidectomy. Limited mastoid cell system filled with granulation tissue. Large atticoantral cholesteatoma.

Table IV *Group IV Sr–85 uptake over 20 %*

Patient	Age	Sex	Sr–85 ( )	Clinical condition, X-ray examination	Surgical findings
1 L. R.	42	♂	21	Chronic secretory mastoiditis with intact ear drum. The mastoid air cell system large and cloudy	Simple mastoidectomy. The mastoid cells contained serous fluid and granulation tissue.
2. R. N.	15	♀	22	Acute mastoiditis in large mastoid air cell system.	Conservative treatment.
3. R. K.	15	♀	27	Acute mastoiditis in large mastoid air cell system.	Conservative treatment

positive percentage if the uptake in the right ear was greater than the accumulation in the left one, and negative in the contrary case So a symmetrical uptake was recorded as 0 per cent (Fig. 1)

The counts measured were of such magnitude that the measuring error due to statistical variation was less than 3 %

The radiation load to the patient from the isotope doses administered was less than 0.5 rad to the bones.

Table V *Group V Sr-85 uptake greater on the opposite side*

Patient	Age	Sex	Sr-85 (%)	Clinical condition, X-ray examination	Surgical findings
1 A. H.	32	♀	−7	Chronic discharging myringitis in the right ear The left ear had suffered from chronic discharge in childhood The mastoid processes were small, partly sclerotic but with normal air content.	Antrotomy and tympanotomy Both antrum and tympanum were normal. No signs of infection in the mastoid air cell either

RESULTS

The measurements are tabulated with the corresponding clinical conditions and the operative and X-ray findings.

The uptake of Sr-85 was increased on the side of the inflamed ear in all but 3 cases. A symmetrical uptake was recorded in 2 cases. No inflammatory changes in the mastoid processes were seen in either of these 2 cases in the X ray examination one of them was found also on operation to have a normal, air-containing mastoid air cell system In one case the uptake of Sr 85 was greater on the side of the healthy ear This patient had chronic granulating inflammation of the auditory meatus and the tympanic membrane. The diagnosis was not confirmed until the operation as the clinical condition was difficult to establish because both the mastoid air cell systems of the patient were shattered and sclerotic as a consequence of a childhood history of otitis

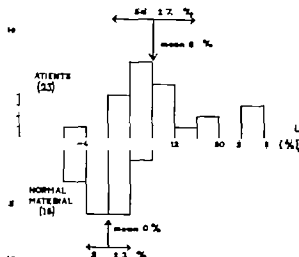


Fig 1 Sr-85 uptake (U) in normal ears and in ears with inflammatory process (patients).

$U = \frac{-c_s}{c_s} \times 100\%$ (patients)

$U = \frac{c_r - c_l}{} \times 100\%$ (normal material)

counts in inflamed ear
c_s counts in healthy ear
counts in right ear
− counts in left ear

The mean uptake value in the control material was 0% and the standard deviation 3 7%

The difference between the patients with ear inflammation and the control patients was statistically highly significant ($p < 0.01$) (Fig. 1)

The results were practically unchanged in the counts made 2–14 days later the count decreased, but the ratios were nearly the same.

CONCLUSIONS AND DISCUSSION

The results obtained warrant the following conclusions

(*a*) The uptake of strontium was increased on the side of the inflamed mastoid process. As the uptake was still increased 2 weeks after injecting the tracer the higher count cannot be due solely to the stimulation of circulation in the inflammatory area but must also be caused by increased calcium metabolism.

(*b*) The strontium uptake was especially strong in acute inflammation of the mastoid process.

(*c*) Inflammation in the extensive mastoid air cell system caused a strong uptake of strontium.

(*d*) The method did not give definite results in cases with a small mastoid air cell system (shattered, sclerotic air cell system) or in cases with sequelae of chronic otitis media.

The isotope method seems to offer a completely new approach for studying the mineral metabolism of the mastoid process and, in this way the activity on the inflammatory process. However, no definite claims about its value in clinical use can be made on the basis of our study though it seems likely that the method could be refined for some measuring techniques. The use of a pulse height analyser would reduce the background disturbances. The results might be clearer if the count were made a few days after administration of the isotope. In addition, the reliability of the results could be improved by keeping the size of the measuring field as closely commensurate as possible to the region of the mastoid process, the Sr-85 scintimetry method of Bauer (1968) may be applicable in this field, too. Sr-85 is perhaps not the most suitable isotope for measurement of the activity of both soft tissue and osseous inflammation. Tc-99m may provide more distinct contrasts, as has been shown in studies of rheumatoid arthritis (Rekonen & Holopainen 1968). But then the disturbing effect of the salivary glands may be difficult to eliminate.

ZUSAMMENFASSUNG

Die Aktivität der Entzündung des Warzenfortsatzes wurde bei 23 Patienten in Übereinstimmung mit dem Prinzip gemessen, dass radioaktives Strontium ebenso wie Kalzium in den entzündlichen Prozessen des Knochens metabolisiert wird. Das Vergleichsmaterial bestand aus 16 Personen ohne Ohrenkrankheiten. Die Gammastrahlung von Sr-85 wurde durch äussere Messung gesondert in den beiden Warzenfortsätzen gemessen. Die Resultate von den entzündeten Warzenfortsätzen wurden mit denen von den gesunden verglichen. Der Unterschied zwischen den Patienten mit der Mastoiditis und dem Vergleichsmaterial war statistisch signifikant. Am intensivsten war die Sr-85 Ansammlung bei den Patienten mit einer aktiven Entzündung der pneumatischen Räume des Mastoids. Die Ansammlung war schwächer bei den Fällen mit kleinen sklerotischen Warzenfortsätzen oder narbigen Residuen der Mittelohrentzündung. Die Anwendung der radioaktiven Isotope ist eine neue Methode zur Forschung des Mineralstoffwechsels im Mastoid, aber die angewandte Methode müsste vervollkommnet werden, ehe sie in die klinische Anwendung gebracht werden kann.

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THE HISTOCHEMISTRY OF DARK CELLS IN THE VESTIBULAR LABYRINTH

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Abstract Dark cells in the vestibular labyrinth from colored guinea pigs w re studied with histochemical methods for number of various enzyme systems. High enzyme activities were observed in the dark cells. Their histochemical reactions were present dif fusely throughout the entire cytoplasm. The dark cells displayed almost the same degree of enzyme ac tivities as cells of the stria vascularis. Presumably these cells may be engaged in high energy-consump-tion with the possible purpose of secretion and/or absorption of endolymph.

Since Iwata (1924) proposed a secretory func for the vestibular labyrinth of the bat, has been accumulating to support this in the various species (Wersäll, 1956 Bairati & Iurato, 1960) However the specific epithelial structures lining the wall of the vestibular apparatus which take part in the secre tion or absorption of endolymph is still disputed.

Recently electron microscopic studies have demonstrated that dark cells and the planum semilunatum might play a role in the produc tion or absorption of endolymph because they are morphologically analogous to the kidney parotid gland, choroid plexus, ciliary body and the stria vascularis (Kimura et al., 1964 Dohl man 1965)

The purpose of this study is to observe histochemically the activity of various enzymes in the dark cells of the vestibular labyrinth of guinea pigs, and to discuss the possibility of a secretory or absorptive function in these cells.

MATERIAL AND METHODS

Thirty colored guinea pigs weighing approximately 250 g were used in this study After decapitation under light ether anesthesia, their inner ears were immediately removed *in toto* The tissue blocks, except for oxidative enzymes, were fixed in 4% neutral formol-calcium or 10% neutral formalin solution for 24 hours at 4 C

All tissues were then decalcified for 7 to 10 days at 4 C with a 10% EDTA solution at pH 7 45 (Balogh, 1962). The decalcified tissue blocks were frozen on dry ice, mounted and cut serially at 10 μ with a rotary microtome in a cryostat (−30 C) The sections were mounted on clean cover-glasses, thawed slowly dried at room temperature for 2 to 3 min, then incubated in one of the substrate solutions designed for demonstration of the activity of the following enzymes: diphosphopyridinenucleotide diaphorase (DPNH-diaphorase), triphosphopyridinenucleotide diaphorase (TPNH diaphorase) lactic dehydrogenase, malic de hydrogenase, succinic dehydrogenase, glucose 6-phosphate dehydrogenase, alkaline and acid phosphatases leucine and alanine amino-peptidases, adenosine triphosphatase (ATPase), non-specific esterase, β-glucuronidase and N-

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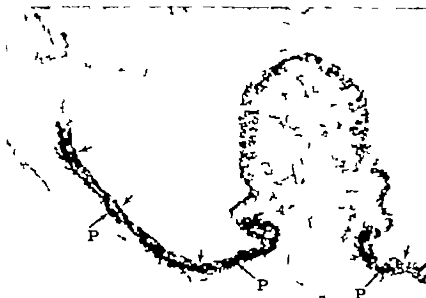


Fig. 1. Frozen section of the horizontal crista ampullaris. The intense DPNH diaphorase activity is seen diffusely throughout the entire cytoplasm of dark cell epithelial lining (*arrow*) which closely follows the pigment cell layer (*P*) in the underlying connective tissue. The remainder of the ampullar wall show very faint enzyme activity. × 190.

acetyl-β-glucosaminidase. Alcian blue staining was used to demonstrate acid mucopolysaccharides (Wanger & Shapiro, 1957). The techniques used for the demonstration of oxidative enzymes in the inner ear were identical to those employed by Nomura & Balogh (1964 *a*). The details of the techniques for the demonstration of hydrolytic enzymes are described in Barka & Anderson's handbook (1963). Controls for the histochemical reactions were made by incubating sections in media from which the respective substrate had been omitted. All histochemical reactions were terminated by fixing the sections in 10% formalin. Finally, the slides were mounted with glycerine jelly for light microscopic examination.

RESULTS

It was possible to distinguish the dark cell layer from other cell layers lining the ampullar or other vestibular wall since the zone of the dark cells follows fairly closely the distribution of the pigment cell layers in the vestibular labyrinth (Kimura et al., 1964; Kimura, 1969).

All enzymes except the non-specific esterase and alkaline phosphatase showed moderate to intense activity in the dark cells.

Striking activity of DPNH-diaphorase, TPNH diaphorase, glucose-6-phosphate dehydrogenase, lactic dehydrogenase and malic dehydrogenase was seen in the dark cells which showed diformazan deposition. Diformazan deposits in the dark cells were diffusely distributed throughout the cytoplasm, whereas in the sensory cells of the cristae ampullares, maculae utriculi and sacculi, cells of the planum semilunatum and transitional cells they were concentrated in the supranuclear portion of the cytoplasm (Figs. 1, 2). Strong to moderate activity of succinic dehydrogenase was also shown in the dark cells, but its activity diminished in the sensory epithelial cells. In the dark cells this histochemical reaction was observed througout the entire cytoplasm. In contrast, the sensory cells showed weak dye deposition in the supranuclear and perinuclear areas of the cytoplasm. There was little histochemical reaction in the transitional cells and the cells of the ampullar or other vestibular walls (Fig. 3). Moderate to strong activity of glutamic dehy-



Fig 2 Glucose-6-phosphate dehydrogenase activity is intense in the dark cell epithelium (*arrows*) of the posterior crista ampullaris. (*P*) pigment cell layer ×140.



Fig 3 Strong succinic dehydrogenase activity is demonstrated in the dark cells (*arrows*) of the horizontal crista ampullaris. The sensory cells contain relatively slight amounts of diformazan. (*P*) pigment cell layer ×130.

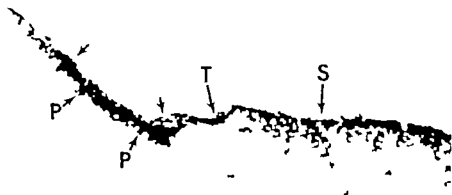


Fig. 4. Azo dye deposits are diffusely distributed throughout the cytoplasm of dark cells (*arrows*), whereas marked acid phosphatase activity is seen in the upper portion of sensory epithelial cells (*S*) and transitional cells (*T*). The rest of the ampullar wall fails to show any enzyme activity. Nuclei are stained with methyl green. (*P*) pigment cell layer. × 170.

drogenase was also found throughout the cytoplasm of the dark cells.

The histochemical distribution pattern of acid phosphatase, β-glucuronidase and N-acetyl-β-glucosaminidase activity was basically similar. Amorphous or fine granular red azo dye was deposited diffusely in the cytoplasm of the dark cells, but in the sensory epithelial cells of the cristae ampullares and maculae utriculi and sacculi, the enzyme activity was intensely concentrated in the supranuclear cytoplasm. In comparison with the sensory cells, the transi-

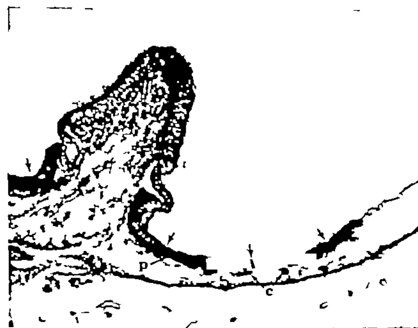


Fig. 5. Membranous ATPase is strongly demonstrated in the dark cells (*arrows*) and capillaries (*C*) underneath the dark cell epithelium. Sensory epithelial cells and transitional cells show considerable activity in the apical cytoplasm. (*P*) pigment cell layer. × 160.



Fig 6 Non-specific esterase activity is feeble in the dark cell epithelium (*arrows*), while sensory cells and transitional cells show moderat to strong activity (*P*) pigment cell layer. × 200

tional cells and cells of the planum semilunatum contained fewer dye granules, which were distributed mainly in the supranuclear portion of the cytoplasm Other cells of the ampullar r vestibular wall showed a very faint reaction g. 4).

Membranous ATPase activity was strongly demonstrated in the dark cells and walls of the capillaries underneath the dark cell epithelium A considerable degree of ATPase activity was also observed mainly at the surface and apical areas of the sensory epithelial cells, transitional cells and cells of the planum semilunatum (Fig. 5)

Alkaline phosphatase activity was restricted to the capillaries underneath the dark cell epithelium including those of the entire membranous labyrinth, all hairs and infranuclear areas of sensory cells of the cristae ampullares and maculae utriculi and sacculi. None of this enzyme was detectable in the dark cells, transitional cells and cells of the planum semilunatum. The non-specific esterase showed feeble activity throughout the cytoplasm of the dark cells, whereas this enzyme was strongly demonstrated in the sensory epithelial cells, less moderately in the transitional cells and the cells of the planum semilunatum, especially in the supranuclear portions (Fig. 6)

Strong activity of leucine- and alanine aminopeptidases was diffusely distributed in the dark cells as well as the transitional cells, cells of the planum semilunatum and sensory cells. The distribution pattern of both enzymes was almost similar Cells of the vestibular wall other than the aforementioned areas showed weak activity of these enzymes (Fig. 7).

The dark cells as well as the sensory cells, transitional cells and cells of the planum semilunatum showed weak staining with acid mucopolysaccharides. All connective tissues underneath the vestibular epithelia and gelatinous membranes covering the maculae and cristae were remarkably stained.

The control sections failed to show any histochemical deposits.

DISCUSSION

Under the light microscope, the planum semilunatum is difficult to distinguish from the dark cell epithelium Kimura et al. (1964) and Dohl-



Fig. 7. Intense leucine aminopeptidase activity is present in the dark cells (arrows), transitional cells (*T*) and sensory epithelial cells (*S*). The vestibular nerve fibers and connective tissues underneath the dark cell epithelium show considerable histochemical reaction. (*P*) pigment cell layer. × 180.

man (1964) have shown that the planum semilunatum can be distinguished from the dark cells because they differ in fine structure. The term planum semilunatum must be applied to the two half-moon shaped areas located in the lateral walls of each ampulla at either end of the crista (Steifensand, 1835). Dark cells are not restricted to the basal area of the crista, but rise up to form a high, complete ring around the utricular opening. They are thus continuous with a similar epithelium from the non-macular portion of the utricle. This dark cell epithelium is also located on the canal side in the form of a wide U band with its opening facing dorsally towards the canal, discontinuous from the utricular side (Kimura et al., 1964. Kimura 1969).

Most of the enzymes applied in this study showed high activity in the dark cells as well as the sensory epithelial cells of the cristae ampullares, maculae utriculi and sacculi, transitional cells and cells of the planum semilunatum as compared with other vestibular epithelial cells.

The dark cells have abundant oxidative enzymes whose reactions result mostly in mitochondrial and partially in extra-mitochondrial formazan.

The dark cells are capable of utilizing anaerobic as well as aerobic glycolysis for energy production and can couple hydrogen with molecular oxygen via the cytochrome system.

It has been stated that the secretory cells of the salt glands in lower animals are characterized by the presence of unusually abundant mitochondria and high levels of oxidative enzymes (Abel & Ellis 1966).

The fine structure of dark cells also shows a close similarity to that of the cells of salt-secret

ing glands. There are numerous finger like projections with many mitochondria at the cell borders and even deep within the cytoplasm which suggest a fluid transporting function.

The fine structure of dark cells also resembles the marginal cells of the stria vascularis in a simple form. Each has a system of complicated folded membranes in the basal portion of the cytoplasm (Nakai & Hilding, 1968 Kimura, 1969) The intense activity of oxidative enzymes has been demonstrated in the stria vascularis (Vosteen, 1961 Nomura & Balogh, 1964 *b*), which has been regarded as the site of endolymph production (Rüedi 1951 Smith, 1957), or absorption (Rauch, 1963) or both (Ruska & Rauch, 1967)

In the present study the dark cells showed almost the same degree of oxidative enzyme activity as the stria vascularis.

The dark cells have remarkable lysosomal enzymes such as acid phosphatase, β-glucuronidase and N acetyl-β-glucosaminidase which are probably associated with such cell functions as phagocytosis, pinocytosis, intracellular digestion or secretion in various organs (Novikoff, 963) In the inner ear Ishii & Balogh (1966) ve demonstrated strong activity of these lysosomal enzymes in the stria vascularis, outer sulcus cells, spiral ligament behind the spiral prominence and epithelium of the limbus, where secretory or absorptive functions have been previously considered to take place (Rüedi 1951 Smith, 1957 Voldrich, 1967)

The biological significance of alkaline phosphatase has not been well understood. In the cochlea of the guinea pig most alkaline phosphatase activity is localized in the walls of capillaries and arterioles (Nomura & Hiraide, 1968) and in the efferent nervous system (Hiraide, 1970) However no alkaline phosphatase activity was shown in the dark cells, but marked activity was detectable in the endothelial cells of blood vessels below the dark cell epithelium. It is worth noting that no alkaline phosphatase activity is observed in the salt-secreting glands except in the blood vessels surrounding the secretory epithelial cells (Abel & Ellis, 1966)

Histochemical evidence for an active proteolytic process in dark cells is provided by the demonstration of marked aminopeptidase activity in these cells. Presumably the protein molecules or their breakdown products eventually pass into the capillary network underneath the dark cells as described in epithelial cells of the mid portion of the endolymphatic sac by Ishii et al. (1966)

Membranous ATPase is the most important enzyme involved in fluid transportation. Using the electron microscope, Nakai & Hilding (1968) have shown ATPase activity on the surface of the folded membranes of these cells as well as of the stria vascularis. In the present study strong ATPase activity was noted in the dark cells. Presumably ion and water transport may take place on the finger-like folded membranes at the cell borders and deep within the cytoplasm of dark cells.

While the function of the non-specific esterase has not been ascertained, its major localization in various organs suggests that it may be related to regulation of tissue size, detoxication and general lipid-ester metabolism (Ballantyne & Bunch, 1967) It seems, however that the dark cells do not require much non-specific esterase for maintenance of their own cell functions because they contain less of this enzyme than other cell components of the maculae and cristae.

Mucopolysaccharides have been reported on the surface between or within the secretory cells of several salt-secreting organs (Abel & Ellis, 1966) However acid mucopolysaccharides showed weak staining in the dark cells. It is interesting to note that the stria vascularis also reacted slightly with acid mucopolysaccharides as Schätzle & Mūsebeck (1963) have reported.

There was some histochemical similarity between the epithelium of the planum semilunatum and the transitional cell epithelium suggesting that their functional roles may be the same Their ultrastructures are also similar (Kimura et al., 1964) In many respects the degree of enzyme activities in these two epithelia is al-

most similar to that of the dark cell epithelium. However the localization of enzyme activities in the epithelium of the planum semilunatum and transitional epithelium differed from that of the dark cell epithelium generally the histochemical reactions were seen diffusely throughout the cytoplasm of dark cells while they were especially localized in the upper portion of the cells of the planum semilunatum and transitional cells. Accordingly considerable functional differences might exist.

It is apparent from the results of the present study and the characteristic findings of previous ultrastructural studies (Smith, 1956 Kimura et al., 1964) that the dark cells have higher activities of various enzymes and large numbers of mitochondria which produce high energy Dark cells might contribute to the secretion and/or absorption of endolymph similar to the stria vascularis.

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ZUSAMMENFASSUNG

Dunkle Zellen im vestibulären Labyrinth von farbigen Meerschweinchen wurden mit histochemischen Methoden für zahlreiche verschiedene Enzymsysteme studiert. Hohe Enzymaktivität wurde in den dunklen Zellen observiert. Ihre histochemischen Reaktionen waren über das ganze Cytoplasma weitverbreitet. Die dunklen Zellen stellten fast dieselben Enzymaktivitätsgrade wie die Zellen der Stria vascularis aus. Vermutlich sind diese Zellen bei grossem Energieverbrauch mit möglicher Absicht der Absonderung und/oder der Aufsaugung der Endolymphe beschäftigt.

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AN ENQUIRY ON THE MORPHOLOGICAL CHARACTERISTICS AND POSSIBLE CHANGES WITH AGE IN THE OLFACTORY REGION OF MAN

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Abstract Methods used in this enquiry are described, the significance of their combined use in investigational procedure is discussed. Surface studies show the relationship of the peripheral olfactory neurones to mosaic formed by the free surfaces of the supporting cells. A marginal ring in the olfactory vesicle is identified and it is shown to consist of basal bodies that give rise to olfactory cilia. Pathological alterations of the olfactory cells are observed and their extent may be assessed by these methods. The normal cellular pattern and zonal distribution of the nuclei of the supporting and sensory cells f the olfactory epithelium is demonstrated in man and it is shown to be lost with age. Other characteristics relating to structure, vascularity and pigmentation of the olfactory epithelium are described. Observations on the mucosal changes with age are recorded.

From a morphological standpoint the olfactory epithelium lacks the transparency and sterile protective environment of other specialized sensory epithelia. The neuro-sensory elements are sensitive to damage and in man the olfactory epithelium is prone to pathological alterations at an early age (1–2 years) (Naessen, 1970 *c*) For obvious reasons human material is seldom available from suitable young subjects and freshly fixed human tissue is nonetheless difficult to obtain at any age.

Macroscopic methods, dissection techniques and methods used in conventional histology in studies of the nasal mucosa have been relatively neglected, research is mainly directed towards ultrastructure

A simple and direct method of visualization of the olfactory organ (epithelium) in man has been described (Naessen, 1970 *a*) Fine dissection techniques and other methods for the examination of the olfactory epithelium are described in this paper Observations are recorded to provide data and to illustrate the advantages and potentialities of the techniques used.

Structural changes in the olfactory region of man with age or disease have received little notice Observations on the cellular and structural organization of the olfactory epithelium in human fetuses and infants are presented and a comparison drawn with observations made on olfactory epithelia in later life

MATERIAL

This consists of nasal mucosae obtained from the olfactory clefts (olfactory regions) of 7 human fetuses (12–24 weeks) 3 infants, 2 children (17 months and 2 /3 years) and 13 human adults with ages 30 years to 65 years. In none were there clinical signs of intranasal or intracranial disease.

Guinea pigs and rabbits were also used for comparative studies.

Some Basic Considerations

Cross sections of olfactory epithelia of man and other mammals are characterised by a peripherally placed, non-nucleated zone consisting



Fig 1 Olfactory epithelium. Guinea pig. "Surface shaving" showing mosaic formed by the free surfaces of the supporting cells and the distribution of the sensory elements (olfactory vesicles) along its inter stitial planes. Vesicles show marginal rings and central dots (*arrows*): former lie on a higher optical plane than those displaying central dots. Phase contrast micrograph 900.

the distal parts of the supporting cells and the peripheral rod-like extensions of the cell bodies of the sensory cells. A sero-mucinous blanket bearing the surface structures or super structure (Naessen 1970 *b*) of the olfactory epithelium is superimposed. This part of the epithelium is relatively transparent and to an extent may be studied to advantage in suitably prepared epithelial surface shavings. The rest of the olfactory epithelium, however is nucleated and more extensive and thus not suitable to this latter method of investigation. Sections may then be obtained from embedded tissue for examination by phase contrast and electron microscopy

METHODS

Soon after death the olfactory region is treated in situ with a 3% solution of glutaraldehyde buffered with 0.075 M sodium cacodylate, pH 7.3 (Naessen 1970 *c*). The ethmoid bone bearing olfactory epithelium is then excised off the facial skeleton, immersed in a chilled solution of 2% osmic acid buffered with Veronal acetate at pH 7.2 and allowed to fix for 2 hours. It is then immersed in 70% ethanol. The olfactory cleft is splayed and the two histological parts of the nasal mucosa identified (Naessen, 1970 *a*) "surface shavings" and whole thickness blocks of mucosa may then be obtained as specimens from chosen sites

Surface shavings for phase contrast microscopy

By fine dissection (using a fine iris knife and watchmaker's forceps) an epithelial "surface shaving" is obtained. This includes the non-nucleated peripheral zone of the olfactory epithelium and its superimposed transparent fluid blanket. The specimen is carefully mounted flat

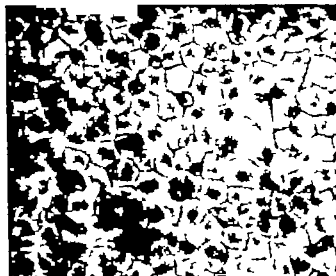


Fig 2 Olfactory epithelium. Human fetus, 22 weeks. "Surface shaving" showing surface mosaic formed by the free surfaces of the supporting cells; olfactory vesicles are few. Phase contrast micrograph ×800.

in glycerine on a slide and a coverslip applied. It is then examined by phase contrast microscopy. By simply focusing down onto varying optical planes through the thickness of this relatively transparent region of the epithelium a stereoscopic impression of the shape, position and relationship of the structures on the surface and intracellular components can be obtained.

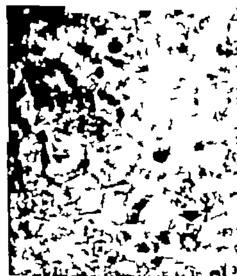


Fig 3 Olfactory epithelium. Guinea pig. "Surface shaving" showing an osmiophilic marginal ring in the olfactory vesicle (*arrow*). The group of vesicles in the upper left corner of the field lie on a higher optical plane. Phase contrast micrograph ×800.

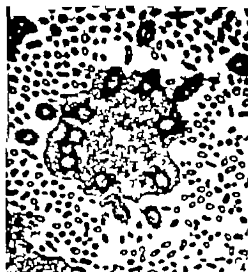


Fig 4 Olfactory vesicle. Human adult, 27 years. Electron micrograph shows osmiophilic marginal ring composed of a set of 8 basal bodies (of olfactory cilia). The vesicle cut tangentially to the free surface of the olfactory epithelium is surrounded by numerous microvilli of adjoining supporting cells. ×20 000

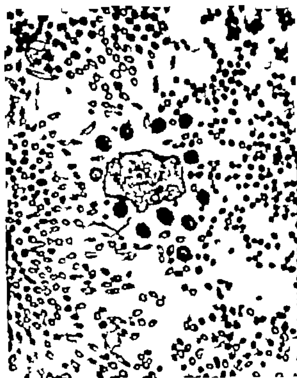


Fig 5 Receptor surface of the olfactory epithelium. Child, 2 / years. Tangential section cut through the radial arrangement of cilia of an olfactory vesicle, Ol t the surface. Their arrangement and relationship to a large number of the microvilli of 4 supporting cells is shown. Electron micrograph 15 000.

Embedding for phase contrast and electron microscopy

By embryological dissection techniques (Iris knife and watchmaker s forceps) a piece of mucosa is resected from its underlying bone or cartilage so that when isolated, its identity and orientation with reference to the parent mucosa and to the region as a whole can be maintained during its preparation for examination under higher resolutions. Resection is carried out in 70% ethanol in a Petri dish placed under an operating microscope. Following dehydration in ethanol the specimen is embedded in Epon. Sections (1–2 μ) are then cut with an ultramicrotome in any required plane and examined by phase contrast.

The procedure allows cutting of ultrathin sections for examination in the electron microscope.

OBSERVATIONS

In man, rabbit and guinea pig the olfactory vesicles appear as small rounded refractile or vesicular bodies and as a general rule are observed to lie singly in the interstices and corners of a mosaic formed by the polygonal contour of the free surfaces of the supporting cells (Figs. 1 2) The vesicles are seen to contain an osmiophilic "marginal ring"[1] (Fig. 3) This ring is formed of basal bodies (Fig. 4) that give rise to olfactory cilia. The cilia are strongly osmiophilic and display a radial arrangement (Fig. 5) Their short straight course can be traced by simply focusing and thus altering the optical plane of a phase contrast microscope. When followed proximally the cilia are seen to converge onto their "marginal ring" of origin in the vesicle.

An osmiophilic central dot (Fig. 1) corresponding in appearance to that described by Le Gros Clark (1956) as an argentophil spot has also been observed. It lies on a lower optical plane than that of the "marginal ring and appears within the cytoplasm of a peripheral rod-like extension of a sensory cell at the surface Its nature is unknown.

The free surface of the supporting cell in the olfactory region as seen by a phase contrast microscope normally appears smooth. In inflammatory conditions of the nasal mucosa the supporting cells have been observed to bear cilia (Fig. 6) Histopathological modifications and alterations of the cells on the surface of the mucosa can be easily assessed and their extent determined

Whereas the nasal mucosa of the respiratory part of the nose is structurally channelled (Naessen, 1970 *e*) that in the olfactory region is studded with buds (Fig. 7) The latter are, however not true sensory buds akin to those subserving taste, but bud-like appearances produced by crypts leading to the intraepithelial ducts of Bowman s glands With age, these appearances become less conspicuous for sub-

[1] This was first seen by Le Gros Clark (1956) in rabbits, using silver stains, but De Lorenzo (1957) was unable to confirm these observations.



Fig 6 Olfactory epithelium. Guinea pig. Surface shaving showing the development of cilia by the supporting cells. Most of the olfactory vesicles have disappeared few are seen in the left half of the field Some supporting cells can be clearly identified individually Phase contrast micrograph 800

sequent to losses of its sensory cells, the olfactory epithelium is reduced in thickness and becomes flat.

Figures 8 and 9 show the light microscopical appearances of the cellular organization of olfactory epithelia of the experimental mammals and man. Peripherally but at some distance from the free surface lies a single layered zone of oval nuclei of the supporting cells. Basally there exists a single layered zone of basal cell nuclei. Between the two zones lies a multilayered middle zone of round nuclei contained within the cell bodies of the sensory cells of olfactory neurones. This pattern of nuclear organization is generally found to obtain throughout the olfactory epithelia examined

In degenerating epithelia, however there occurs a disturbance in the zonal distribution of the supporting and sensory cell nuclei (Figs 10, 11). An intermingling of nuclei results as the supporting cells retract and conform to the general reduction in thickness of the epithelium following receptor neurone losses. The regular monolayered zone of supporting cell nuclei is reestablished following the total disappearance of the receptor neurones. The basal cells remain relatively unaffected. Such epithelia are seen to consist solely of supporting and basal cells (Fig. 12) The supporting cells retain a reasonably normal appearance in the face of considerable sensory cell degeneration The general surface architecture (mosaic) is still present even though most of the olfactory vesicles have disappeared.

It has been shown that the olfactory margin in man is regular and that early in life (1–2 years) alterations occur and impart to it a



Fig 7 Olfactory mucosa. Human fetus, 24 weeks. Section shows pseudosensory bud-like structure. The thickness of the epithelium is determined mainly by the complement of sensory cell nuclei. Compare figures 10 11 12, 13 Phase contrast micrograph 540.

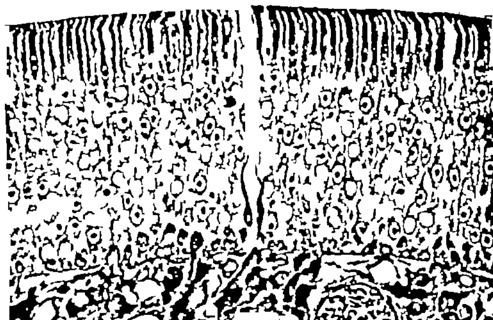


Fig. 8 Olfactory epithelium. Guinea pig. A broad zone of sensory cell nuclei with conspicuous single nucleoli lies between a layer of basal cells and peripheral layer f supporting cell nuclei possessing ... than one nucleolus. Clear rod-like, extensions ... the sensory cells are interposed between the dark distal parts of the supporting cells in a non-nucleated zone forming the outer quarter of the epithelium. Olfactory vesicles are recognizable in the clearer basal half of a relatively narrow seromucinous zone (blanket). An intra epithelial duct of a Bowman gland is showing. Phase contrast micrograph 650.

characteristic irregularity (Naessen, 1970 *c*) Whether the demarcation line between the olfactory and respiratory areas is regular or irregular the junction between the two territories as seen in histological sections is always abrupt (Fig 13) such junctions show no intermixing of cell types. An intermixture of cells, however does occur in diseased and degenerating epithelia where dying neurones lie along epithelial cells undergoing ciliated transformation (Fig. 14) Moreover artefacts simulating olfactory vesicles occur in the respiratory and olfactory parts of the nose and are particularly conspicuous in areas adjoining the olfactory margin (Figs. 15 16)

In infancy and early childhood the olfactory epithelium is vascular (Figs. 17 18) The lamina propria upon which it rests is rather loose and cellular a basement membrane intervenes (Fig. 19) The basal cells form an indefinite layer of cells. Blood capillaries encroach upon the basal layers of the olfactory epithelium and are in intimate association with the perikarya of the sensory cells. Some vessels are seen invested in sleeves of connective tissue derived from the lamina propria. With age there occurs regression of these intraepithelial vessels and the olfactory epithelium from a morphological point of view thereafter remains avascular A definite layer of basal cells forms and the latter now rest upon an apparently "amorphous basement membrane (Figs. 10



Fig 9 Olfactory epithelium. Human fetus, 22 weeks. Section showing the regular zonal distribution of nuclei. Supporting cells possess dark elongated nuclei and sensory cells possess clear round or oval nuclei basal cell layer indefinite. Peripheral rod-like extensions of the sensory cells and their terminal ends or olfactory vesicles are sparse and inconspicuous. The supporting cells are tufted (microvilli) some show protrusions t the surface. Blood capillaries are intimately related to the basal layers f the epithelium. Phase contrast micrograph 650.



Fig 10 Olfactory epithelium Human adult, 30 years. Section shows oval (supporting cells) and round (sensory cells) nuclei and definite layer of basal cells. A depletion of the sensory cell population is apparent, dark degenerating cells are present. Supporting cell nuclei have in parts lost their regular zonal rrangement and the supporting cell cytoplasm (peripherally) shows an accumulation f pigment granules. Olfactory vesicles are seen t the surface. The supporting cells are tufted. Phase contrast micrograph 650.



Fig. 11. Olfactory epithelium. Human adult, 45 years. Section shows a marked depletion of the sensory cell population. Degenerating sensory cell nuclei are dark, show frayed outlines and are indented. Surviving neurones are clear group is seen at the right end of the micrograph. The supporting cells are pigment-laden. Their nuclei have lost their zonal distribution and mingle with the sensory cell nuclei. A few olfactory vesicles are seen at the surface. A basement membrane is conspicuous. Phase contrast micrograph 650.

11 12, 13) the basement membrane, seen electromicroscopically is interposed.

Pigment-granules constitute a conspicuous feature of the supporting cells of olfactory epithelia in adult man (Figs. 10, 11 12, 13 20) These are made even more conspicuous by their increased numbers in the olfactory epithelia of the aged. These granules are main- present in the cytoplasm of the more central parts of the supra-nuclear portion of the supporting cells (Fig. 20). The pigment masses have an irregular outline and a highly heterogeneous interior consisting of dense bodies of varying size. No pigment granules have been seen in the olfactory epithelia of fetuses, infants and young children nor in the young of the experimental mammals.



Fig. 12. Olfactory epithelium. Human adult, 61 years. Section shows a total sensory cell depletion. Epithelium consists solely f supporting and basal cells. The supporting cell nuclei have lost their longated or oval appearance and are definitely round. A Bowman's gland shows marked distension of its lumen, *B*. Phase contrast micrograph 650.



Fig 13 Olfacto-respiratory junction. Human adult, 51 years. Note its sharp delineation, no intermixing of cell types is seen on either side. The olfactory epithelium (to right of junction) is in an advanced state of degeneration. Sensory cell depletion is almost total. Phase contrast micrograph 720

DISCUSSION

A macroscopic method for the identification of the olfactory epithelium in man and other mammals has been described (Naessen, 1970 *a*) The method is useful in the precise localization of the olfactory epithelium and its preservation for examination in greater detail by complementary methods used in this study Data derived from observations made provide basis for experimental and quantitative studies in assessing vulnerability of this sensory organ to damage.

The method of studying cellular detail by optical scanning" through cells of sensory epithelia similar to that used here was first described by Neubert (1950, 1952) in studies on the cochlea. The method was later modified and used by Engström et al (1966) in studies on the inner ear

Fine dissection techniques are used to obtain surface shavings from freshly fixed specimens of nasal mucosa. Observations derived from these are easier to interpret than those made on tangential sections from embedded tissue. Specimen preparation time is greatly reduced. Silver stains used to trace the course of neural elements in this sensory organ are liable to produce artefacts. They form precipitates on the surface and along interfaces. No single silver stain is reliable in reproducing effect Osmic acid has the advantage of not only preserving closely the form of the living structure but of reacting differentially with cell components. Consequently these specimens are less liable to alterations and are less prone to show artefacts Image contrast is greatly improved when specimens, so fixed, are examined in conjunction with phase contrast optics

Phase contrast micrographs 7–13 17 18 shown in this text are those of sections obtained from specimens embedded in Epon These sections yield a better resolution than the much thicker celloidin sections in common use. They



Fig 14 Olfactory epithelium. Guinea pig. Section shows dying neurone with its centrioles (basal bodies) pursuing a retrograde course. Adjoining supporting cells undergo ciliated transformation. This electron micrograph was obtained from the specimen shown in Fig. 6 above. Electron micrograph × 20 000.

permit a clear study of the deeper parts of the olfactory epithelium and observations can be correlated with those derived from surface studies described above. One use of a combination of these methods provides evidence that the sensory elements of the olfactory epithelium are not all represented on the surface. Alternatively a surface mosaic devoid of olfactory vesicles does not necessarily imply that the epithelium is devoid of sensory cells. These observations are important in studies on the development, degeneration and regeneration of the olfactory neurones.

An intermingling of supporting and sensory cell nuclei in man is often illustrated in current textbooks of anatomy and histology in descriptions of the olfactory epithelium. These appearances are common and as shown in this study are characteristic of olfactory epithelia in states of degeneration.

There are various forms that olfactory sense cells can adopt at the surface (rod-like truncated, spheroidal, ellipsoidal) and variations do occur in the pattern of their internal distribution of optically visible components. Technical factors contribute to producing these inconsistencies. It is also possible that in their reaction to the environment the cells are capable



Fig 15 Ciliated respiratory epithelium. Child, 17 months. Section shows non-ciliated epithelial cell flanked by two ciliated cells. It bears microvilli and is about to extrude its contents of mucus. This physiological deformity may simulate an olfactory vesicle when seen in the light microscope. Electron micrograph 16 000.

of adopting various forms and cause distortion of the normal arrangement of their intracellular organelles. Such activity is arrested at the moment of fixation to give the variety of static pictures seen in electronmicrographs. These variations in fixed preparations can then not be interpreted as artefacts nor a variety of structures of a different kind.

Artefacts simulating olfactory vesicles may have led light-microscopists in the past to believe in an intercellular mixture of olfactory and respiratory elements the olfactory margin was difficult to define The electron microscope reveals the true nature of these artefacts which in the majority of instances are due to staining precipitates and physico-physiological deformities of the epithelial cells that may occur at the free surface of the nasal mucosa in the respiratory and nonrespiratory parts of the nose (Figs. 15 16)

Except in the fetus and infant the peripheral olfactory neurones have not been seen to contact blood vessels but have supporting cells interposed. Electron microscopy demonstrates a close structural and metabolic relationship between the olfactory neurones and these latter cells (Naessen, 1970 *d*) With age, the vessels in the epithelium regress. The lamina propria adjoining the basement membrane looses its cellularity and becomes apparently amorphous. This then constitutes the basement membrane of classical histology An increase in the amorphous ground substance of the lamina propria may limit diffusion



Fig 16 Olfactory epithelium. Guinea pig. Section shows the fronded appearance of physiological deformity f supporting cell with heightened metabolic activity (Naessen, 1970 *d*). Electron micrograph 14 000.

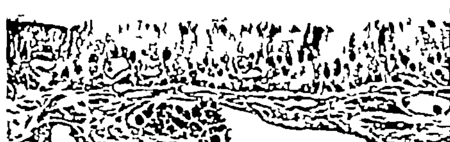


Fig 17 Olfactory epithelium. Child, 17 months. Section from near the olfactory margin showing blood capillaries *within* the epithelium. Phase contrast micrograph 620

Whereas membrane bounded bodies of degenerated material in the young experimental mammal are commonly found in the infranuclear part or vascular pole of the supporting cells (Naessen, 1970) the pigment particles in adult man appear to accumulate and form deposits mainly in the supranuclear cytoplasm of these cells. Pigment aggregates increase in number with advancing age as in other cells of the body particularly those of stable and permanent character One possible explanation the heterogeneity of this class of residual bodies is that they contain debris of neurone degeneration (extraneous) or remnants of degenerating organelles (intrinsic) incorporated into lysozomes during the normal turnover of cell components. The accumulation of these residual bodies in the olfactory supporting cell with age may signify an inability on the part of the cell to deal effectively with the excretion of its own metabolic wastes as well as with the products of neuronophagic activity (Le Gros Clark, 1956 Naessen, 1970 *d*)

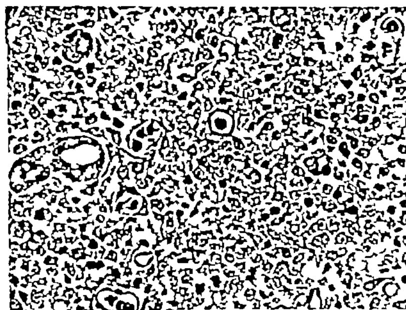


Fig 18 Olfactory epithelium. Infant, 11 months. Tangential section showing the intimate relationship of the blood capillaries to the sensory cell perikarya and their axons in the basal layers of the epithelium. Phase contrast micrograph 700.

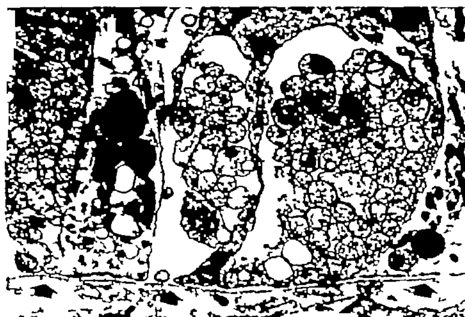


Fig 19 Olfactory epithelium, Guinea pig. Section showing intraepithelial bundles of naked receptor axons held by infra nuclear cytoplasmic extensions of two basal cells. The basal "foot of supporting cell is interposed it contains an ppreciable amount f lysozomal bodies. Part of another "foot is seen at the extreme right f the micrograph. All est upon basement membrane (*arrows*). Electron micrograph 20 000.



Fig 20 Olfactory epithelium. Human adult, 51 years. Section shows pigment granules in the supporting cells. Note the heterogenous appearance of the granules. The supporting cells are bound together by junctional complex consisting of terminal bar (*arrow*) and at least 6 button-like desmosomes lying in row on curve below the arrow Electron micrograph 25 000

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ZUSAMMENFASSUNG

Die Methoden, die in dieser Untersuchung benutzt wurden, werden beschrieben. Die Signifikanz ihres kombinierten Gebrauchs in diesem Untersuchungsverfahren wird besprochen. Flächenstudien zeigen die Beziehung der peripheren olfaktorischen Neuronen zu einem Mosaik, das durch die freie Fläche der versorgenden Zellen geformt wird. Im olfaktorischen Muster wurde ein marginaler Ring identifiziert, der aus basalen Körpern besteht, die wiederum die Ursache zu olfaktorischer Cilia sind. Pathologische Verschiedenheiten der olfaktorischen Zellen werden beobachtet, und ihre Ausdehnung könnte durch diese Methoden festgestellt werden. Das normale Zellenmuster und die zonale Verteilung der Nuclei der versorgenden und sensorischen Zellen des olfaktorischen Epithels beim Menschen werden gezeigt, und es ist erwiesen, dass sie sich mit dem Alter verfieren. odere Charakteristika in Bezug auf Struktur Vasrisation und Pigmentation des olfaktorischen pels werden beschrieben. Beobachtungen bei den osen Veränderungen im Alter werden besprochen.

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RECENT TRIALS IN THE TREATMENT OF MAXILLARY SINUS CARCINOMA, WITH SPECIAL REFERENCE TO THE CHEMICAL POTENTIATION OF RADIATION THERAPY

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Abstract Four hundred cases of M xillary Antrum Carcinoma, which were treated at Osaka University Hospital during 1957–68, were reviewed. Special emphasis was laid on the prospective controlled trial to evaluate the chemical potentiation of radiotherapy In the cases treated up to the end of 1966, external radiotherapy followed by radical operation was applied in general. The 5-year survival rates of these were 30% for the combined group, and 13% for the group receiving radiotherapy alone. Although results have been showing improvement due to technical advancement, including high energy radiotherapy the 5-year survival rate of the total cases treated between 1961 and 1966 was less than 30% Since the beginning of 1967 controlled trial has been under way to evaluate the chemical potentiation of radiotherapy by arterial infusion of 5-Fluorouracil, Cyclohexanol succinate or Ametobepazone, or by oral administration of Cyclohexanol succinate. Of these, there has been found improved ecurrence-free rates in the arterial infusion groups of 5-Fluorouracil and of Cyclohexanol at 6 to 24 months observation. The difference of the results between 5-Fluorouracil group and the radiotherapy alone group was statistically significant at 6 months and at 12 months. Any difference in the 1 to 2-year survival figures has not been found, possibly due to subsequent successful surgery

Carcinomas of the maxillary sinus are fairly common in Japan. The incidence of the disease is nearly as great as carcinomas of the larynx, but the overall 5-year cure rate still remains less than 30% This is a report of approximately 400 cases of histologically proven Maxillary Antrum Carcinoma, which were treated at Osaka University Hospital between 1957 and 1968 During this period, external radiotherapy was the first step in the treatment in every case, followed by surgery if this were indicated. Since the beginning of 1967 chemical potentiation has been added to radiotherapy using intra-arterial infusion technique, as a controlled trial.

This study was supported by grants from the Welfare Ministry of J pan.

Classification

The TNM system of the UICC, which has been widely applied to head and neck cancer still remains to be established for the classification of Maxillary Antrum Carcinoma. In this study the lesion has been classified into four stages on an anatomical basis, according to the tentative TNM system proposed by Sakai & Hamasaki (1967) (Fig. 1)

T 1 indicates a tumor localized within the maxillary sinus, without radiological evidence of bony involvement.

T 2 indicates a tumor causing destruction of the bony wall, with the external periosteum remaining intact and the surrounding tissue not invaded but possibly compressed.

T 3 indicates a tumor infiltrating deeply into the surrounding tissue through penetration of the external periosteum.

T-4 indicates a tumor extending into the base of the skull, the nasopharynx or the maxilla of the opposite side

Concerning metastases, the UICC classifica-

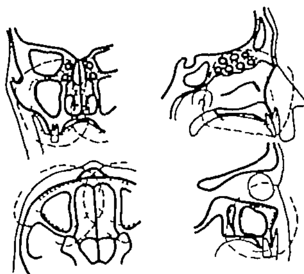


Fig. 1 Classification of maxillary antrum carcinoma by tumor extension.

tion for carcinoma of the buccal cavity was applied as follows:

N Regional lymph nodes
- N0 no palpable lymph nodes
- N1 movable homolateral lymph nodes
- N2 movable contralateral or bilateral lymph nodes
- N3 fixed homolateral or bilateral lymph nodes

M Distant metastases
- M0 no evidence of distant metastases
- M1 distant metastases

Results of the cases treated during 1957–1966

During the period of 1957–66 there were some changes in the method of the 200 kVp X ray apparatus by Cs-137 and Co-60 gamma ray equipment. Until the end of 1960 a tumor dose of 4 000 to 5 000 rad was given in 5 weeks using a 200 kVp machine through 4 to 6 portals. During the period of 1961–1962, a dose of 5 000 to 6 000 rad was delivered in 6 weeks using a Cesium-137 Unit with one anterior and two lateral opposing fields. Since 1963 a dose of 7 000 to 8 000 rad has been

Table I *3-year survival rates of maxillary antrum carcinomas*

Stage	II	III	IV	Total
RT + OP				
1957 ~ 62 (200 kVp)	17/36 47	10/32 31	1/5 20	[illegible]8/73 38
1963 ~ 65 (Co–60)	10/23 43	5/8 63	—	15/31 48
RT				
1957 ~ 62 (200 kVp)	8/24 33	2/47 4	0/17 0	10/88 11
1963 ~ 65 (Co–60)	5/18 28	6/31 19	2/15 13	13/64 20

given in 8 weeks using a Cobalt-60 unit with 3 portals as in case of Cs-137. In the past, until the end of 1966 en block surgical resection of the maxillary antrum if possible was performed within 4 to 6 weeks after the completion of radiotherapy with the agreement of the patient.

The survival figures of the cases treated during this period are shown in Table I and Fig.

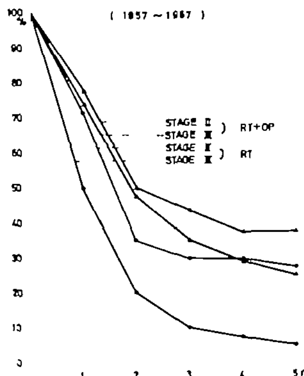


Fig. 2 Survival figures of maxillary antrum carcinomas (1957 1967).

2. The group which received surgery appeared to have a better result than the group which received radiotherapy only even when we take into account the fact that this is not a randomized study and there could be a real possibility that poor risk cases may have been deliberately excluded from surgery The difference in the results is most remarkable in the advanced Group T 3 As can be imagined, there has been some improvement in the results since the use of Cobalt-60 Unit has become routine still the locally advanced cases of Maxillary Antrum Carcinoma appeared difficult to control with radiation alone

Chemical potentiation since 1967
(a controlled trial)

At the end of 1966, a controlled trial was planned to evaluate the chemical potentiation to radiotherapy Beginning in January 1967 cases of Maxillary Antrum Carcinoma have been randomized by even and odd case number on admission to the study Patients of poor general condition and those having regional or distant metastases were excluded, but the age has not been taken into account. Concerning the cases to be discussed in this paper all of the treatable cases of N-0 and M-0 were involved without considering the stage by T classification. However it was decided to exclude very early cases of T 1 and very advanced cases of T-4 at the end of 1968 Stratification according to stage is now under consideration. Case distribution by age and stage in each group is shown in Figs. 3 and 4 A randomly allocated group of radiotherapy cases, without chemical potentiation, was considered as the control.

Every case in this program was planned to receive a tumor dose of 8 000 rad in 8 weeks using a Cobalt 60 Unit and a wedge pair technique. Prior to the beginning, or within the first week of radiotherapy Caldwell-Luc Antrostomy was done for drainage and for convenience of examination. Since the program started, the radical operation has not

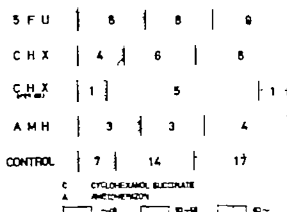


Fig 3 Case distribution by age in each trial group.

been performed until the recurrence or tumor residual was confirmed.

The drugs, which were examined in this pilot study were 5-Fluorouracil (Jesse, 1969 Scotte Doggett et al., 1967 Vermund et al., 1969) Cyclohexanol succinate (Scolari et al., 1965) and Ametohepazone (Nilbe et al., 1967 Tobe et al., 1967) (Fig. 5) The former two drugs have been already fairly often discussed in America and Europe. Ametohepazone is not a drug recognized as a sensitizer but in Japan has been said to be effective for pain relief in advanced cancer patients. Bleomycine, which was recently discovered in Japan and has been expected to be an effective drug for the control of squamous cell carcinoma, was also intended to be examined. It has

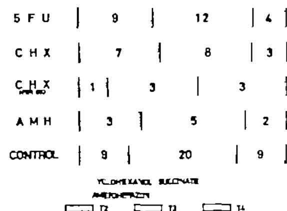
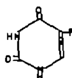
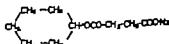


Fig 4 Case distribution by T-classification in each trial group.

5 F U



CYCLOHEXANOL SUCCINATE



AMETOHEPAZON

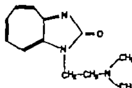


Fig 5 Molecular formula of 5-Fluorouracil, Cyclohexanol succinate and Ametohepazon.

been rather sporadically used, but not in this program.

The drugs were administered by the continuous intra arterial infusion method using a portable roller pump through a superficial temporal artery or superior thyroid artery Cyclohexanol succinate was examined by oral administration as well as by infusion. The periods during which each of the various drugs was used are shown in Fig. 6 The dose of each drug administered is shown in Table II. The dose has been modified depending on the side effects, especially the mucosal reaction. The drug was intended to be administered for as

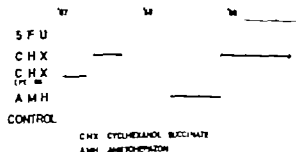


Fig 6 Period of trial in each drug

Table II *Given dose of each drug*

	5-FU	CHX	CHX (per OS)	AMH
mg/kg/day	5 ~ 10	10 ~ 20	30 ~ 40	1 ~ 2
g/day/person	0.25 ~ 0.5	0.5 ~ 1	1.5 ~ 2	0.05 ~ 0.1
Total dose/ period	5 g/ 4 ~ 7 w	25 ~ 30 g /7 ~ 8 w	90 g/ /60 d.	2.5 ~ 3 g /7 8

CHX = cyclohexanol succinate. AMH = ametohepazon

long as possible during the course of radiotherapy

Results of the cases
of the controlled study since 1967

For the first step of the study it was intended to evaluate the effect of the drugs during the course of radiotherapy from the practical point of view one, from the speed of tumor regression and, two from the mucosal reaction. Needless to say long term results are most important. From our impression during the course of radiotherapy 5-Fluorouracil seems to give faster tumor regression than Cyclohexanol succinate, but the mucosal reaction in cases treated with 5 Fluorouracil tended to develop severely at an early date, which often made it impossible to deliver the planned dose of radiation. Some serious complications were encountered in the 5 Fluorouracil group, which will be discussed later In the Cyclohexanol group, there has not been any difficulty in giving the planned dose of 8 000 rad in 8 weeks.

The results so far obtained at this moment may be best illustrated in the histograms of Figs. 7–11 These histograms show in detail what happened in the individual patient after radiotherapy

In Tables III and IV the survival rate and recurrence free rate of each trial group are summarized by observation period. As is shown in Table III, there has not been seen any difference in the survival rate between any of the chemical potentiation groups and the control group

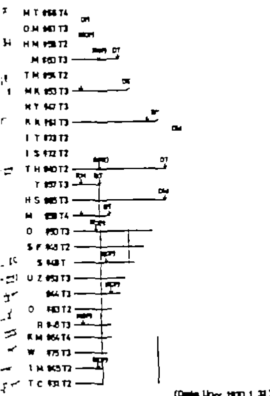


Fig 7 Histograms show what happened in the individual patient after radiotherapy (5-FU infusion group).

Since the radical operation has been performed only on the occasion of tumor residual or recurrence in this program, the recurrence free rate following initial radiotherapy is the most important parameter when the tumor controllability by each trial is considered. In Table IV the best recurrence-free rate appeared in the 5-Fluorouracil group and the second best in the Cyclohexanol arterial infusion group through the observation period of 6 months to 2 years. Of these recurrence-free rates the difference between the 5-Fluorouracil group and the control was statistically significant at 1% level at 6 months observation and at 5% level at 12 months. Since

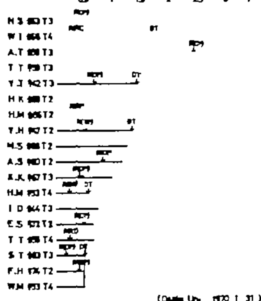


Fig 8 Histograms show what happened in the individual patient after radiotherapy (Cyclohexanol succinate infusion group).

seven cases of the initial trial in Cyclohexanol succinate intra-arterial infusion group revealed surprisingly good results, this was again put into the program for further evaluation as is shown in Fig. 6. The result, while not statis-



Fig 9 Histograms show what happened in the individual patient after radiotherapy (Cyclohexanol oral administration group).

AMETOHEPAZON INFUSION GROUP

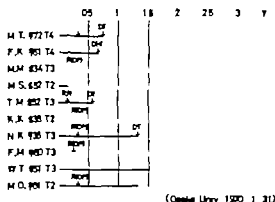


Fig 10 Histograms show what happened in the individual patient after radiotherapy (Ametohepazon infusion group).

CONTROL GROUP

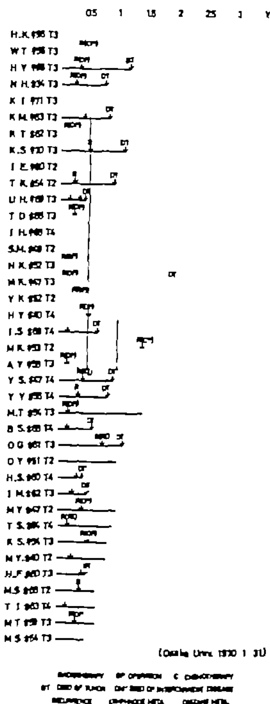


Fig 11 Histograms show what happened in the individual patient after radiotherapy (Control group).

tically significant, still leads us to be hopeful. The cases given Cyclohexanol succinate, orally and the infusion group of Ametohepazone did not behave differently than the control group

The cases under consideration are still limited in number and in observation period It is still uncertain at this time whether the differences, which have been found in the recurrence free rates, still are related to a possible improvement of permanent tumor control or only represent prolongation of the interval between radiotherapy and recurrence.

Needless to say further experience and longer observation are needed. However it may be concluded that the continuous intra-arterial infusion of 5 Fluorouracil or Cyclohexanol succinate during the course of radiotherapy has possibilities of improving the control rate in the Maxillary Antrum Carcinoma.

DISCUSSION

A number of reports have appeared dealing with chemical potentiation of radiotherapy However this type of treatment still has a variety of problems. It remains undetermined whether there "is" or "is to be" any merit in "this combined approach" In addition to that optimization of the dose and mode of administration of the drug still requires refinement

Of the two drugs which appeared effectiv

In this pilot study 5-Fluorouracil resulted in faster tumor regression than Cyclohexanol succinate, however the side effects of 5-Fluorouracil were much more severe than those of Cyclohexanol. It has been our general rule that the drug should be continued as long as possible during the course of radiotherapy without reducing total dose of radiation. At the beginning of the program, in the 5-Fluorouracil study a dose of approximately 250 mg was given each day through the superficial temporal artery or the superior thyroid artery. In some cases of the series, a serious reaction of the skin or mucous membrane made it impossible to give more than 5 000 rad in 5 weeks. In one such case, which had revealed apparently complete tumor disappearence the tumor recurred within 3 months and the subsequent operation failed to salvage the case. In another case, catheterization of the superior thyroid artery with subsequent chemotherapy was followed by paralysis of a recurrent laryngeal nerve on that side. This complication took place when the patient received 7 000 rad in 7 weeks with a total dose of 5 g of 5-Fluorouracil. In spite of having this problem, the patient is now fairly well doing without recurrence after 2 years observation. The voice has shown improvement by compensation of the opposite vocal cord. Since such complications were encountered, the dose of 5 Fluorouracil was reduced up to the level of 100 mg a day and the infusion through a superior thyroid artery has been given up. Although the serious complications have been avoided in later patients, after the schedule of the infusion was modified, the results are still too early for evaluation.

In the Cyclohexanol succinate infusion group, no serious complications have been encountered. Because of the encouraging result of the first series of the study this drug has again been put into the program after an interruption for further evaluation.

The group, oral administration of Cyclohexanol succinate" failed to show any better results, possibly because of smaller concentration in the blood supplied to the tumor.

Cases of Maxillary Sinus Carcinoma often develop a recurrence at a long interval following radiotherapy. Although there has been shown some improvement in control at 6 to 24 months observation, further experience and longer observation are needed before a conclusion can be reached.

Surgery still plays a great role in the management of the cases of recurrence or tumor residue after radiotherapy even in conjunction with chemotherapy. At present, the survival rate of the control group, which has shown nearly the same as the trial group, seems to be supported by the surgically salvaged cases.

Table III. *Crude survival rates of maxillary antrum carcinoma of each trial Group*

(Osaka Univ 1970.1.31)

	0.5 Y	1 Y	1.5 Y	2 Y
5-FU	24/25	13/17	9/14	5/9
(infusion)	96	76	64	56
CHX	17/18	11/11	6/7	5/7
(infusion)	94	100	86	71
CHX	7/7	6/7	5/7	3/7
(per OS)	100	86	71	43
AMH	10/10	7/10	5/9	0/2
(infusion)	100	70	56	0
Control	34/38	20/30	14/23	10/17
	89	67	61	59

CHX cyclohexanol succinate AMH ametobeparon

Table IV *Recurrence free rates of maxillary antrum carcinoma of each trial group*

(Osaka Univ 1970.1.31)

	0.5 Y	1 Y	1.5 Y	2 Y
5-FU	15/25	8/17	6/14	3/8
(infusion)	60	47	43	38
CHX	8/18	4/11	3/7	3/7
(infusion)	44	36	43	43
CHX	3/7	1/7	1/7	1/7
(per OS)	43	14	14	14
AMH	3/10	2/9	[illegible]/8	0/1
(infusion)	30	22	25	0
Control	9/38	7/30	5/23	5/17
	24	23	22	29

$P<0.05$, $P<0.01$

CHX = cyclohexanol succinate. AMH = ametobeparon

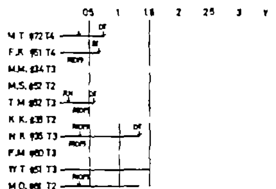


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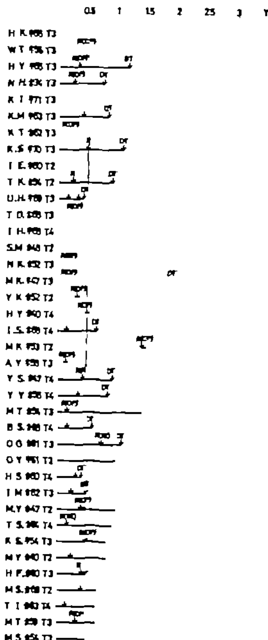


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METHODEN DER QUANTITATIVEN EIWEIßBESTIMMUNG IM MENSCHLICHEN PAROTISSPEICHEL

A. Skurk, G. Mlynski und K. Fendel

Aus der Universitäts-Hals-Nasen-Ohrenklinik Jena, DDR

(Eingegangen am 1. Juli, 1970)

Abstrakt. Der Wert von 6 Bestimmungsmethoden des Gesamteiweißes im Parotisspeichel wird überprüft. Es handelt sich dabei um vier modifizierte Biuret-Reaktionen, die Lowry-Methode und eine nephelometrische Bestimmung. Dabei ergeben sich sowohl methodische als auch individuelle Abweichungen, die auf eine nicht vollständige Erfassung des Gesamteiweißes bei einigen Methoden hindeuten. Auf Grund der vorliegenden Untersuchungen werden zwei neue Methoden für die Proteinbestimmung im Parotisspeichel angegeben, die den gegenüber Serum und Liquor andersartigen Verhältnissen Rechnung tragen.

Zur quantitativen Bestimmung von Eiweiß im Parotisspeichel werden eine größere Zahl unterschiedlicher Methoden angewendet. Die Lowry-Methode (Daughaday et al., 1952) wird bevorzugt, weil sie für die geringe Konzentration des Speichelproteins die größte Empfindlichkeit besitzt. Wolf & Taylor (1964) haben verschiedene Proteinbestimmungsmethoden auf ihre Anwendbarkeit für Parotisspeichel überprüft. Es wurde festgestellt, daß zwischen den einzelnen Methoden und den verschiedenen Proben signifikante Differenzen bestehen. Es erscheint deshalb angezeigt, nach Methoden zu suchen, die sich durch eine einfache Handhabung bei ausreichender Empfindlichkeit sowie durch hohe Spezifität und Haltbarkeit der Reagenzien auszeichnen. Im Folgenden werden zwei neue Untersuchungsverfahren (modifizierte Biuret-Bestimmungen) dargestellt und die Ergebnisse mit denen anderer Methoden verglichen.

METHODE

Der Parotisspeichel wurde von gesunden Personen mit einer von Sprindrichova & Sprindrich (1968) beschriebenen Modifikation der Curby-Kapsel isoliert. Stimuliert wurde der Speichelfluß durch orale Gaben von Ascorbinsäure oder Zitronensäure. Die Speichelproben wurden bei 2–4 Grad aufbewahrt und vor der Eiweißbestimmung zentrifugiert.

1. *Biuret-Bestimmung bei 334 nm*

Die von Bürgi et al. (1967) beschriebene Methode der Liquoreiweißbestimmung mit einem modifizierten Biuret-Reagenz im UV wurde ohne Änderung für die Speichelproteinbestimmung übernommen.

2. *Biuret-Bestimmung nach Eiweißfällung*

Aus 0,5 ml Parotisspeichel wird mit 1,0 ml eiskalter 0,66 N Perchlorsäure das Eiweiß ausgefällt und der abzentrifugierte Niederschlag in 1,0 ml Biuret-Reagenz (Richterich, 1968) auf gelöst. Die Messung der Extinktion erfolgt nach 30 min gegen Biuret-Reagenz als Leerwert bei 546 nm.

3. *Nephelometrische Bestimmung mit einem modifizierten Exton-Reagenz*

Nach Ausfällen des Proteins mit Sulfosalicylsäure/Natriumsulfat wird die scheinbare Extinktion der trüben Lösung bei 470 nm bestimmt (Germand & Hajek, 1966).

4. *Lowry-Methode*

Wir verwendeten die von Zak & Cohen (1961) beschriebene Gebrauchslösung, die sich durch größere Beständigkeit auszeichnet. 4,0 ml dieser

Tabelle I *Gesamteiweiß in mg/100 ml bei 14 Personen bestimmt mit zwei Biuret Methoden, der Lowry Methode und der Ausfällung mit Sulfosalicylsäure*

Nr	Biuret UV (334 nm)	Biuret/ $HClO_4$-Fällung (546 nm)	Sulfosalizylsäure Fällung (470 nm)	Lowry (578 nm)
1	79,–	49,3	58,8	77 6
	161	93 4	116	178
3	125	43,5	51,0	191
4	118	56,0	83 6	208
5	65,6	26,8	43,7	79,0
6	218	133	101	306
7	98 1	36,6	28,5	14_
8	19	168	152	370
9	264	81,3	97 4	291
10	16	74 7	77,9	195
11	156	55 1	38,9	185
12	222	142	183	205
13	179	101	140	183
14	89,9	40 4	42,1	80,5

Gebrauchslösung werden mit 0 1 ml Parotisspeichel gemischt und nach 10 min mit 0,5 ml Phenolreagenz versetzt. Das Ablesen der Extinktion erfolgt nach 30 min bei 578 nm gegen einen mit NaCl-Lösung analog hergestellten Leerwert

5 *Direkte Biuret Bestimmung bei 546 nm*

Die von Maurer (1968) beschriebene Schnellbestimmung des Gesamtproteins im Liquor wurde für die Speicheleiweißbestimmung in folgender Weise geändert.

Reagenz: 0,3 g $CuSO_4$ 5 H_2O in 5 0 ml H_2O Auffüllen mit 60 Tl. 2 N NaOH/40 Tl. Äthylenglykol auf 100 ml.

Für die Bestimmung werden 0,5 ml Biuret Reagenz mit 0,5 ml Speichel gemischt und die Extinktion nach 15 min gegen den aus 0,5 ml Biuret Reagenz und 0,5 ml NaCl-Lösung hergestellten Leerwert bei 546 nm bestimmt.

6 *Biuret Bestimmung nach Acetonfällung bei 546 nm*

Durch Zusatz von 0,5 ml Speichel zu 2,0 ml 3% Essigsäure in Aceton wird das Protein ausgefällt (Komarov & Stavraky 1940) Nach Abzentrifugieren und Auflösen des Rückstandes in 1 0 ml Biuret Reagenz wird die Extinktion bei 546 nm gemessen.

Bei allen Methoden wurde ein durch Verdünnen eines käuflichen Kontrollserums hergestellter Standard verwendet, dessen Eiweißgehalt nach Kjeldahl bestimmt worden war

ERGEBNISSE

Tabelle I gibt das für 14 Proben nach den Methoden 1–4 ermittelte Gesamtprotein in mg/100 ml an.

Die angegebenen Werte wurden aus 3 Meßwerten berechnet. Für die Proben 9 10 und 11 wurden 20 Bestimmungen in Serie gemessen, um Aussagen über mögliche Korrelationen gewinnen zu können. Dabei ergibt der *t* Test signifikante Unterschiede zwischen den einzelnen Methoden, ausgenommen davon sind nur die Werte der Probe 10 für die Methoden 2 und 3 hier besteht kein signifikanter Unterschied. Der Variationskoeffizient ist für die UV Biuret Methode mit 2 % am niedrigsten Die Variationskoeffizienten der anderen Methoden liegen zwischen 5 und 8% Korrelationen bestehen zwischen den Methoden 1–4 nicht, da die Angabe einer Regressionsgeraden nicht möglich ist.

Die Ergebnisse der für die Speichelprotein

Tabelle II *Gesamteiweiß in mg/100 ml bestimmt mit der Biuret Schnellmethode und nach Acetonfällung*

Nr	Biuret Schnellmethode (546 nm)		Biuret Acetonfällung (546 nm)		Lowry (578 nm)	
1	198		210			
2	227		247			
3	316		391			
4	212		248			
5	75,4		77,0			
6	137		152			
7	40,5		45,0			
8	774		288			
9	495		530			
10	268	274	286	278	250	218
11	404	405	480	474	333	320
12	260	275	282	790	19	246
13	168	175	172	187	153	145

bestimmung geänderten Biuret Methoden 5 und 6 gibt Tabelle II an. Die Proben 10–13 wurden nochmals mit der Folin-Methode nach Lowry verglichen.

Für die Proben 1–4 wurde durch 20 Bestimmungen in Serie der Variationskoeffizient ermittelt. Er liegt für die Schnellmethode bei 3.4% für die Acetonfällung bei 4.0%. Die Reproduzierbarkeit beider Methoden (Proben 10–13) ist gut. Das Lambert-Beersche-Gesetz ist bis zu einem Eiweißgehalt von 500 mg/100 ml erfüllt.

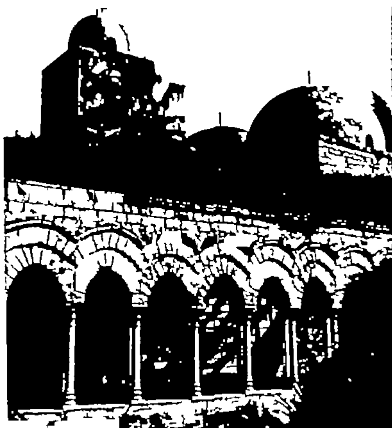
Die Fällungsmethode ergibt signifikant höhere Werte als die Biuret Schnellmethode und als die Lowry Methode. Zwischen den beiden Biuret-Methoden besteht eine Korrelation ($r = 0.999$), der Unterschied ist signifikant ($p < 0.001$).

DISKUSSION

Die Biuret Reaktion erfüllt im Hinblick auf Spezifität und Haltbarkeit der Reagenzien die gestellten Anforderungen. Besonders für die Bestimmung des in geringer Konzentration vorliegenden Parotisproteins ist aber die Empfindlichkeit zu gering. Die Messung beim Absorptionsmaximum des Cu-Biuret Komplexes im UV (Methode 1) steigert zwar die Empfindlichkeit, erhöht aber auch die Störungen durch niedermolekulare Verbindungen. Eine Messung im Sichtbaren ist nach vorheriger Fällung (Methode 2) möglich. Die Ausfällung mit Perchlorsäure liefert im Vergleich mit der UV-Methode sehr viel niedrigere Werte, die durch unvollständige Fällung des Proteins bedingt sind. Die Ausfällung mit Sulfosalizylsäure (Methode 3) ist in der Durchführung am einfachsten. Im Gegensatz zur Eiweißbestimmung im Liquor versagt sie im vorliegenden Fall, weil die Ausfällung unvollständig ist und auch durch Zusatz von Harnstoff (Krause & Seidler 1969) der Kolloidzustand nicht stabilisiert werden kann. Die Ergebnisse der Fällungsmethoden 2 und 3 liegen im gleichen Bereich, ohne daß eine Korrelation zwischen ihnen besteht. Die farbbildende Reaktion der Lowry-Methode (4) hängt neben dem Vorliegen der Peptidbindung auch vom Gehalt an den aromatischen Aminosäuren Tyrosin und Tryptophan ab. Dies führt zwangsläufig zu einer Abhängigkeit der Farbintensität von der Art des Proteins. Da auch die Technik dieser Methode anspruchsvoll ist, halten wir sie zur Routinebestimmung von Parotiseiweiß für wenig geeignet.

Die Proteinkonzentration im Parotisspeichel hängt nach Dawes (1969) sowohl von der Flußmenge als auch von der Dauer der Stimulation ab, so daß die Angabe von Normalwerten ohne Berücksichtigung dieser Parameter nicht sinnvoll ist. Möglicherweise ändert sich während der Sekretion nicht nur die Proteinmenge, sondern auch deren Zusammensetzung. Die elektrophoretische Auftrennung von Parotisspeichel gesunder Probanden zeigt innerhalb der einzelnen Fraktionen beträchtliche Schwankungen. Hinzu kommt im Vergleich zu Serum und Liquor der höhere Anteil an den Enzymen Amylase und Lysozym, der auch in weiten Grenzen schwanken kann. Die qualitativen Unterschiede der einzelnen Proben bedingen die Schwierigkeiten bei der quantitativen Bestimmung des Proteins, denn keine der Methoden 1–4 scheint in jeder Probe den Totalproteinwert anzugeben. Fehlerhaft sind besonders die Resultate der Fällungsmethoden, da hier offenbar die Enzyme nicht erfaßt werden. Schon durch die Untersuchungen von Windeler & Shannon (1966) war bekannt, daß die meisten Fällungsmethoden für die Bestimmung des Reststickstoffes ungeeignet sind, da sie in der Mehrzahl das Eiweiß nur unvollständig entfernen. Die Ausfällung mit Essigsäure/Aceton (Methode 6) nach Komarov & Stavraky (1940) ist zwar für die Reststickstoffbestimmung nicht geeignet, da das organische Lösungsmittel die Aufarbeitung erschwert; das Eiweiß wird aber vollständig gefällt, ohne daß beim angegebenen Verhältnis niedermolekulare Verbindungen ausfallen. Da nur der Niederschlag benötigt wird, stört das organische Lösungsmittel nicht. Ein anderer Weg wird mit Methode 5 beschritten. Gegenüber der UV Methode (1) wird die Erhöhung der Eiweißkonzentration im Meßansatz durch Zugabe einer größeren Menge Speichel bei gleichzeitiger

Almqvist & Wiksells
BOKTRYCKERI AKTIEBOLAG
UPPSALA 1971



INTRODUCTION

E. Borghesan

Chers Collègues, Mesdames, Messieurs,
Je remercie beaucoup les Membres du Collegium qui ont choisi Palerme pour la session Orlas du 1970 et qui sont venu ici. Je remercie aussi les Dames, lesquelles rendent aimable la réunion avec leur présence. Mes salutations très cordiales aux nouveaux Membres du Collegium, ils prennent le flambeau de l'Olympiade scientifique et sont l'espoir de l'évolution de l'Oto-Rhino-Laryngologie. A tous les collègues je souhaite bon travail et sejour heureux.

Meine Herren, Meine Damen,
Den Mitgliedern des Collegiums und den gnädigen Damen, danke ich bestens, für die Teilnahme an dieser Versammlung des Collegium Orlas. Den neuen Mitgliedern, sage ich willkommen und alle Kollegen wünsche ich eine gute Arbeit und einen angenehmen Aufenthalt.

Dear Colleagues, Ladies and Gentlemen,
I wish to thank the Members of the Collegium for having chosen Palermo for the Orlas reunion and for coming here in such numbers and to extend a special welcome to the Ladies whose presence will lighten the seriousness of our meeting.

To the President of the Sicilian Government and to the President of the Regional Assembly to the Mayor of Palermo to the Presidents of the Local Councils my gratitude for their cooperation in this cultural event.

It is a great pleasure to have here the world's

most illustrious representatives devoted to otolaryngology and pure science

I feel bound to recall, with sadness but with gratitude, those Members who have departed for ever For them let us have a moment s silence.

Today we are opening the series of the meetings of the seventies on this occasion I should like to express the hope that we may all meet again at the end of the decade, to record the progress of our science I assume this progress will be considerable in view of ever improving research techniques, and, above all, as a result of the work of the younger Members who have recently joined our Collegium. They inherit a special Olympic torch, the Olympics of science, which is a contest without victors or vanquished, representing an act of faith in the continuity of an ideal whose scope is the search for truth in human biology and pathology In welcoming our new Members, I express the wish that they may attain the ideals they have set themselves.

Last August, in Mexico City when you did me the honour of entrusting me with the organization of this meeting in my City I wondered I should be equal to the task but I was mediately encouraged when I realized that our choice was a sign of friendship and I accepted with pleasure because one s friends are tolerant of such failings as cannot always, for one reason or another be avoided

Today you are assembled on an island which differs from all others in its geographical situation, the fertility of its soil and its climatic conditions. All these elements have left their mark on its complex history

Sicily is surrounded by a sea, which links three continents with their great, ancient civilizations and in the course of centuries this sea has been used by many nations who one after the other were tempted to conquer this island, whose life history dates from the early paleolithic age

Prehistoric man has left graphites of great expressive power in caverns on Mount Pellegrino. In the eighteenth century B C Sicily was populated by the Sicani and the Siculi later on they mixed with the Phoenicians and with the Aegean peoples. These last have given rise to a superb civilization, while the Punics, after conquering the west of Sicily have left only ruins. In the third century B C. the Punics were driven away by the Romans and towards the fourth century A.D., after the raids of the Vandals and the Goths, the Romans left the dominion to Byzantium. This dominion was then kept by the Arabs, by the Normans and the Swabians to them succeeded the Angevins, the Aragoneses, the Spaniards, the Sicilian Barons and at last the Bourbons. In 1860 Sicily was united to the reign of Italy and since 1947 after the establishment of the Republic, Sicily became a Region with a special statute

All down the ages, this island has produced men of keen intellect among them one thinks of Archimedes for mathematics, Giacomo da Lentini, Cielo d Alcamo Meli, Quasimodo for poetry Antonello da Messina and Novelli for their painting, Bellini and Scarlatti for their music, Verga, Cesareo and Pirandello for literature Michele Amari for history Juvara, Carnalivari and Basile for architecture, Antonello Gagini, Rutelli and Civiletti for sculpture, Giacomo Serpotta who excelled in the art of stucco, Pitrè in philology and folklore Cannizzaro in chemistry and Maiorana in physics.

Palermo, which the Phoenicians called ZIZ" which meant flower and the Greeks called Panormo which meant all of it a port was involved for many centuries in the fate of the island and went through periods of struggle and decadence especially under the Vandals, and periods of great splendour especially under Fredric II, who made of the City one of the greatest cultural centres of Europe In this period the Italian language began to substitute Latin. Today Palermo has many museums, fine churches, ancient palaces, an ancient Academy of Science and Letters, an Academy of Fine Arts and a University The City with its six hundred thousand inhabitants, is dominated by Mount Pellegrino and stretches in the Conca d'Oro the Golden Valley"

The course of time and the numerous conquerors leave their mark on the character of nations, and the people of Sicily by reason of their ancestral experiences, are sceptical and firmly convinced that everything remains unchanged even when everything seems to change. These experiences have contributed to the formation of a character whose prevailing features are: pride, distrust of the unknown, a strong attachment to the family and a great capacity for friendship Thus the dual concept of family-friendship has become a refuge and comfort in the human relations, which are in this country always showy and colourful In Sicily welcoming relations and friends is a reason for a celebration, in the company of Sun, Air and Earth.

All these elements combine to attack the senses and seem to inhibit action, while stimulating the mind to fantasy contemplation, meditation. In consequence, a conflict arises here between the duty towards scientific tasks and the pleasure of receiving friends. Such conflict can be resolved provided work is considered a necessity of life and not a drudgery

Your attendance at this meeting in Palermo gives me not only pleasure but also the welcome impression that, in addition to the scientific reasons for your coming, you wished to visit this patch of earth for the sake of its own great richness, philosophy and ethics.

This has led me to try to fulfil all your desires and I have tried to bring all the strands together so that Sicania, once Trinacria or Triquetra and now Sicily may remain in your minds as one of your happiest memories. Encouraged by this hope, I wish all a happy stay and to my colleagues a period of profitable work.

I now have the pleasure of showing you, by courtesy of Esso Standard, a panoramic film of Sicily as a land of dreams. Thank you

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AUFBAU UND UMBAU DER KNÖCHERNEN LABYRINTHKAPSEL DES MENSCHEN

G Zechner

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Abstrakt Durch eine entsprechende Entkalkung und spezielle histologische Darstellungsmethoden haben wir Einblick erhalten in den Aufbau der menschlichen Labyrinthkapsel. Die organische Matrix enthält ein dichtes Netz von Lacunen und Kanälchen mit Osteocyten in den verschiedensten Funktionsstadien. Die Grundsubstanz besteht aus einem strähnenartig verflochtenen Fasernetz und einer aus reichlich sauren MPS bestehenden Kittsubstanz. Der Stoffwechsel besteht vorwiegend in einer Umlagerung der Mucopolysaccharide und in einer Änderung ihres Polymerisationsgrades. Umbau zeigt sich im Sinne intralacunärer Resorption und Apposition, morphologischer Umgestaltung und molekularer Umstellung der interglobulären Räume, sowie der Mantelbildung entlang von Gefäßen. Die Labyrinthkapsel ist unseren Untersuchungen nach eine sehr aktive Organstruktur bis ins hohe Alter. Sie erhält sich ihre foetalen Merkmale lange und unterliegt einem sehr langsamen Reifungsprozeß. Sie ist sicherlich kein überalteter Knochen oder ein toter Kalkfelsen.

Die menschliche knöcherne Labyrinthkapsel ist ihrer Entwicklungsgeschichte nach, dem Bauprinzip und der Morphologie der knöchernen Bestandteile entsprechend, ein sehr kompliziertes Gebilde Nach den ersten Untersuchungen Manasses haben vor allem Siebenmann (1894) Mayer (1917) Eckert Möbius (1924) und Bast (1930) ganz wesentlich dazu beigetragen, daß Meyer (1933) seine bis heute morphologisch unwidersprochen gebliebene normale Anatomie der Labyrinthkapsel erarbeiten konnte. Wie schwierig die zu behandelnde Materie ist, haben gerade in letzter Zeit Mißverständnisse deutlich gezeigt, die ihre Ursachen in fehlerhaften Beobachtungen hatten, die darin gipfelten, daß die Labyrinthkapsel als vorzeitig gealtert bzw aus totem Knochen bestehend erachtet wurde. Um Entstehungstheorien der Otosklerose zu untermauern, haben Rius & Mendoza (1961) das Fehlen der Osteocyten in Lakunen und das Vorhandensein von Fetttröpfchen in diesen angeführt. Gussen (1967) vermeinte in den „globuli interossei ein Zeichen von Knochenresorption — aseptischer Nekrose — erkennen zu können. Obwohl schon Meyer (1933) darauf verwiesen hat, daß der Labyrinthkapselknochen nur mit Spezialmethoden untersucht werden kann, bedienten sich nur Engström & Röckert (1962) der Polarisation und des Mikro-Röntgenogramms und Mikro-Radiographie, sowie Chevance (1961) der Elektronen-Mikroskopie und Del Bo (1961) und Ricci (1962) Mucopolysaccharid-Reaktion am foetalen Felsenbein Costa & Covell (1965) Gussen (1968) um nur einige zu nennen, verwendeten immer noch haematoxilin-eosin-gefärbte Präparate zur Grundlage ihrer Beobachtungen. Gerade eine geeignete präparative Technik ermöglichte uns, Einblick zu erhalten in die Biologie der menschlichen Labyrinthkapsel, die offenbar nach dem zweiten Lebensjahr andere Wege geht, als das übrige Skelett. Ein grobmorphologischer Umbau nach dem zweiten Lebensjahr hat nicht statt. Lamellen-Systeme im Sinne osteonartigen Knochens fehlen, eine Knochen-Breccie entsteht nicht. Hauptbauelement der Kapsel ist der Strähnenknochen, der Knorpelreste enthält, wie sie schon Manasse (1897) beschrieben hat. Dies alles war Meyer (1933) bereits bekannt und es bedurfte verbesserter Entkal-

kungsmethoden, besserer Fixierung und moderner histo-morphologischer und histo-chemischer Methoden, um entscheidende Fortschritte zu erzielen.

MATERIAL UND METHODIK

Probleme in der Verarbeitung menschlicher Felsenbeine liegen neben einer guten zeitgerechten Fixierung in einer schonenden Entkalkung. Wir fixieren mit gepuffertem neutralem Formol, wobei noch Formalin ins Mittelohr eingebracht wird. Nicht nur an den Sinnesendstellen machen sich postmortale Veränderungen bemerkbar wir sehen sie ebenso an den Osteocyten und der Grundsubstanz. Schon Remagen & Stolpmann (1962) haben darauf verwiesen, daß postmortale Veränderungen und Säureentkalkung dieselben Schäden setzen, insbesondere an den MPS der Grundsubstanz. Wir entkalken nach Gussen & Donahue (1965) mit EDTA, Versene oder Titriplex und zwar bei einem p_H von 7,35 und einer 0 7 molaren Lösung. Die Vorteile dieser Chelierungs-Methode zeigen sich in der ausgezeichneten Anfärbbarkeit der Präparate und auch in Möglichkeit der Betrachtung im polarisier Licht. Die Entkalkung muß sehr gewebeschonend sein. Weitere Schwierigkeiten ergeben sich aus dem Einbettungsverfahren, welches die Schnittdicke ganz entscheidend beeinflußt. Uns hat sich die Celloidin Einbettung als unersetzlich erwiesen, obwohl 18 μ dicke Serienschnitte das Optimum des Erzielbaren waren. Unseren Untersuchungen liegen siebzig menschliche Felsenbeinpaare aller Altersgruppen zu Grunde. Da die Haematoxilin-Eosion-Färbung — Meyer (1933) hat schon darauf hingewiesen — nur bescheidene Resultate gibt, haben wir nach einer Bindegewebsfärbung gesucht. Bahr & Huhn (1952) haben erst neulich zeigen können, daß gerade die Grundsubstanz das Färbeergebnis bei Bindegewebsdarstellung ganz entscheidend beeinflußt. Beste Resultate erzielten wir mit Pollak s modifizierter Masson-Trichrom-Färbung (Abb 6) Die Beobachtung, daß sich der Strahnenknochen anders färbt, als der lamelläre, hat uns veranlaßt, besonders der Grundsubstanz unsere Aufmerksamkeit zu widmen. Ihre Bestandteile Fibrillen-Collagen und Kittsubstanz Mucopolysaccharide sind chemisch aneinander gebunden. Wir benützten polarisiertes Licht und Mucopolysaccharid-Darstellungen (Abb 2–5) Sicher sollte man nach Lindner (1965) von kombinierten Methoden nie dieselbe Aussagekraft erwarten, als von Einzelnachweis-Reaktionen. Unsere Ergebnisse aber mit der kombinierten MPS Eisen PAS Reaktion nach Mowry waren bei genauester Beachtung der Vorschrift sehr gut, da sowohl saure, als auch neutrale MPS deutlich nachweisbar waren. Auch das Lacunenkanälchen System hat sich sehr übersichtlich dargestellt, sodaß wir die gesamte organische Matrix der Labyrinthkapsel beurteilen konnten.

UNTERSUCHUNGSERGEBNISSE

Formbedingende und formbewahrende Schicht der Labyrinthkapsel ist die mittlere aus Strähnenknochen. Nach innen zu liegt eine endostale Belegschicht, aus Bindegewebe durch primäre Ossifikation entstanden. Die periostale alles umgebende Schicht besteht aus einem Maschenwerk geflechtartigen Knochens, dessen gröbere Faserbündel in der Hauptachse des Felsenbeins gelegen sind. In den Maschen liegt zum Teil Strähnen- zum Teil lamellärer Osteonknochen. Diesen Aufbau erkennt man zum Teil bereits im HE gefärbten Präparat, am deutlichsten in der Schneckenkapsel, weniger in der Bogengangskapsel.

Die endostale Schicht fehlt häufig an der Schneckenspitze und Teilen der Bogengangswand. Die enchondrale Schicht wiederum enthält Knorpelreste es fehlen Kittlinien, die Fasersysteme sind zopfartig mit Knotenpunkten. Die periostale Lage ist teils durch eine Ansatzlinie streckenweise aber auch durch kein morphologisches Detail von der enchondralen getrennt und reicht weit ins Felsenbein hinein. Dieser komplizierte Bau leitet sich aus der Entwicklungsgeschichte her die endostale Schicht

entspricht der General Lamelle die enchondrale entsteht aus einem knorpeligen Block. Die Ossifikation entspricht der an der Epiphyse langer Röhrenknochen, nur fehlt die periostale und endostale Knochenbildung. Knorpelreste bleiben bestehen, da, durch keine Knochenmanschette behindert, eine flächenhafte Eröffnung statt hat. Auf die Knorpelreste wird feinfaseriger embryonaler Knochen abgelagert, wie ihn Gegenbauer auch am Röhrenknochen gesehen hat. Die osteoklastische Modellierung ist kurz und der Zubau erfolgt ohne wesentliche Resorption. Knorpelreste und Gefäßte werden mattenartig umgeben von feinfaserigem und nach Meyer Bast und Weber lamellenlosen Knochen. Hier bleibt die Entwicklung stehen zum Unterschied vom ganzen übrigen Skelett. Die periostale Kapsel entsteht durch direkte Bindegewebsverknöcherung.

Mit Hilfe der Pollak schen Trichrom-Färbung beobachteten wir den geschilderten Aufbau besonders gut und übersichtlich, da sich alle Schichten und die sie aufbauenden Knochenarten verschieden anfärbten (Abb 7) Die endostale Belegschicht erscheint blaßgrün und deutlich durch eine Linie abgesetzt. Die enchondrale Lage zeigt den dunkler grünen, bzw türkisen Strähnenknochen und die globuli ossei in derselben Farbe. Die interglobulären Räume bleiben ungefärbt. Die im HE-gefärbten, tiefblauen Grenzscheiden der Gefäße (Abb 1) sind orange. Die periostale Lage hat ein dunkelblau bis schwärzliches Maschengerüst (Abb 7) in welches nach innen zu türkiser Strähnenknochen und nach außen roter lamellärer Osteonknochen eingelagert ist. Knorpelreste fehlen. Ebenso wie im HE-gefärbten Schnitten sind Osteocyten, Lakunen und Kanälchen schlecht zu sehen.

Die Mucopolysaccharid-Eisen-PAS-Reaktion brachte gerade bezüglich letzterer Strukturen sehr interessante Informationen. Der Aufbau der Labyrinthkapsel spiegelt sich auch in dieser Reaktion wider (Abb 3) Die endostale Lage ist blau angefärbt, die enchondrale im wesentlichen bläulich mit rötlichen Arealen und den tiefblauen Knorpelresten, die periostale Lage blau-rötlich mit deutlich roten Bezirken in der Peripherie. Die Grenzscheiden der Gefäße sind violett-rot gefärbt, ebenso, wie Grenzbezirke um die globuli ossei und einzelne Partien im lamellären Knochen. Besonders auffallend war aber das bläulich gefärbte, im Strähnenknochen sehr dichte Lakunen-Canaliculi-System, welches Osteocyten der verschiedensten Form enthielt (Abb. 8) Sie waren groß, wanzenartig, die Lakune füllend, aber auch spindelig zart mit einem breiten Hof und deutlicher Rouget Neumann scher Scheide

Ein weiteres morphologisches Detail sahen wir insbesondere in der Bogengangskapsel. Um Gefäße denen die Grenzscheiden fehlten, fand sich mantelförmig bläulicher Knochen mit violetten Säumen (Abb 5) Diese Areale scheinen ohne Grenze in Knorpelreste überzugehen. Teile dieser Knochenmäntel färben sich rötlich oder sind vollkommen rot (Abb 6) Haltelinien trennen diese Bildungen vom umliegenden Knochen.

Die Fasersysteme stellen sich am besten im polarisierten Licht dar mit und ohne Kompensator (Abb 2) Die endostale Lage enthält mehr minder ungeordnete, kurze feine Faserbündel. Die enchondrale Lage hat im Strähnenknochen feinfaserige, sich zopfartig verflechtende Systeme, die durch keine Kittlinien unterbrochen sind und Knotenpunkte aufweisen. Die Fasersysteme zeigen weiters eine mattenartige Struktur (Abb 2, 3) deren Strähnen sich, unter dem Gipsplättchen betrachtet, unter 90gradigem Winkel durchdringen (Abb 2) In der periostalen Lage fällt besonders das Lamellen-System des Osteonknochens (Abb. 3) auf, das im deutlichen Kontrast zum kurzfaserigen Bindegewebsknochen steht.

Besprechung der Ergebnisse

Das Knochengewebe ist eine Durchdringungs-Struktur nach Knese (1963), deren verschiedene Gewebskomponenten durch gesonderte Bildungsvorgänge hervorgebracht werden und eigenen Stoffwechselgesetzen gehorchen. Zwischen den Komponenten bestehen topogra-



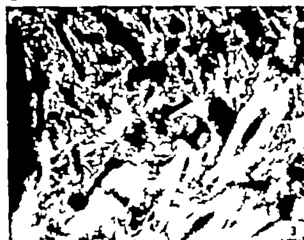
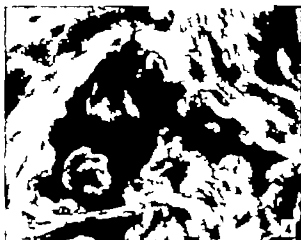





Abb 1 Labyrinthkapsel HE, Übersicht.
Abb 2. Labyrinthkapsel im polarisierten Licht und Glimmplättchen.
Abb 3 Labyrinthkapsel MPS-Eisen-PAS-Reaktion und polarisiertes Licht.

Abb 4 Interglobularer Raum. MPS-Eisen-PAS-Reaktion.
Abb 5 Blaue Mantel und interglobuläre Raume. MPS-Eisen-PAS-Reaktion und polarisiertes Licht.
Abb 6 Rote Mäntel, Pollak's Trichrom.



Abb. 7. Labyrinthkapsel. Grenze enchondrale und periostale Lage. Pollak's Trichrom.

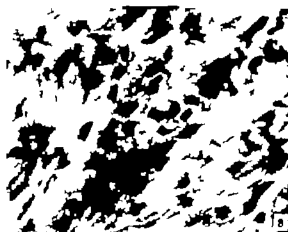


Abb. 8. Osteocyten im Strähnenknochen. MPS-Eisen-PAS-Reaktion.

phische Beziehungen und ein Verhältnis der Abhängigkeit der Mineralien vom organischen Teil. Eger (1963) nimmt sogar an, daß die Mineralisation der limitierende Faktor des Wachstums ist. Die organische Matrix, der uns besonders interessierende Teil des Knochens, setzt sich aus den Osteocyten, welche nach Gibian (1959) Baüd (1962), Lipp (1955) Wassermann & Jaeger (1965) nutritiv formativ und reparativ tätig sind und der hochdifferenzierten, teils stärker teils weniger geformten Grundsubstanz, welche vornehmlich der mechanischen Leistung dient, zusammen. Diese Leistung erbringen nach Knese & Harnack (1962) vor allem die Fasersysteme wobei eine Art Verbundbau anzunehmen ist, bei welchem die Kittsubstanz aus mehr oder minder hochpolymerisierten Mucopolysaccharid-Protein-Komplexen eine wichtige Rolle spielt. Die Kittsubstanz erfüllt nach Gibian (1959) aber auch eine Speicherfunktion für extrazelluläres Wasser aber auch Calcium. All dies gilt auch für das Felsenbein und die menschliche Labyrinthkapsel, wie wir an Hand unserer Befunde zeigen konnten, wodurch wir aber im Gegensatz zu Manasse (1897) Siebenmann (1894) Mayer (1917), Eckert Möbius (1924), Bast (1930) Rius & Mendoza (1961), Fleischer (1957) und Gussen (1968) stehen. Durch MPS-Darstellungen konnte veranschaulicht werden daß das Lacunenkanälchen-System gerade im Strähnenknochen besonders gut ausgebildet ist. Es enthält reichlich Osteocyten und Grenzscheiden verschiedenster Funktionsstufen, bei des nach Wassermann & Jaeger (1965) Hauptwege des Stoffwechsels im Knochen. Sie vermitteln auch, wie Baüd (1962) zeigen konnte, zwischen Kapillaren und interzellulärer Matrix. Diese Wege sind im Strähnenknochen eher kurz. Als weitere Zentren starken Stoffwechsels gelten bei Graumann (1958 1964) und Godard (1951) die basophilen Inseln nach Zawisch (1927) Sie entsprechen den interglobulären Räumen im Strähnenknochen. Wie Lipp (1955) und Zawisch (1927) konnten wir neben der morphologischen Umgestaltung auch die molekulare Umstellung im Sinne einer Depolymerisation an Hand der PAS-positiven Säume zeigen. Gussen (1968) hat dieselben Beobachtungen gemacht. Costa & Covell (1965) glauben sogar eine Abnahme der Knorpelreste feststellen zu können, was Kakizaki & Altmann (1970) nicht sicher widerlegen konnten. Diese Knorpelreste sind nach Lipp (1955) nicht nur Platzhalter sondern auch Energie Depots. Auffallend ist weiters, daß der Strähnenknochen reichlich niederpolymere, saure MPS enthält, die mit Kollagen eine eher lockere Bindung nach Gibian (1959) eingehen, was ein Zeichen gewisser Umreife des

A

Knochens nach Mjör (1962) und v d. Hooff (1964) darstellt und im wesentlichen nur ver kalken, aber nicht mineralisieren, das eine Annahme Gussens (1967–68) und die Untersu chungen Engströms & Röckerts (1962) bestätigen.

Wie an allen übrigen Knochen zeigt sich Stoffwechsel-Aktivität an den Osteocyten selbst an ihrer Gestalt und der Dicke des MPS-Mantels z. B aber auch an der Umgebung, welche zuerst Verlust der Basophilie und dann stark positive PAS-Reaktion zeigt. Dies heißt einerseits Wechsel im Polymerisationsgrad, aber auch Mengenzunahme der MPS nach Graumann (1958–64) Morphologisch äußert sich die Osteocyten-Aktivität nach Lipp (1955) in intralacunärer Resorption, aber auch Apposition. Dies kann — wenngleich am Strähnenknochen selten — zum völligen Zu mauern der Lacunen führen Es entsteht das Bild der Frost schen Mikropetrosis. Echten Umbau findet man am Strähnenknochen nur ausnahmsweise Lineare Apposition tritt nach dem zweiten Lebensjahr in den Hintergrund Knese (1963) sieht aber auch Zuwachs durch Reifung der Komponenten, d. h. Abnahme der MPS zu Gunsten des Kollagens und Zunahme Polymerisationsgrades und durch intraos- Neubildung. Die Flächendichte der Osteo- yten nimmt ab und zwar nicht zuletzt durch Volumszunahme der interzellulären Substanz. Dieser Alterungsprozeß verläuft am Strähnen knochen sehr langsam und dauert bis ins hohe Alter

Morphologisch faßbaren Umbau sahen wir an den interglobulären Räumen und zwar in derselben Form, wie Zawisch (1927) Lipp (1954) und Ruth (1961) wobei uns aber Zu sammenhänge mit den Manasse-Weber schen Mantelbildungen aufgefallen sind. Besonders in der Bogengangskapsel finden sich diese um ein Gefäß gelagerten Knochenneubildungen als blaue gemischte und rote Mantel je nach der Knochenart die sie aufbauen. Schutz vor re gellosem Abbau bilden nach Lipp stark saure Areale, wie es im Strähnenknochen die Knor pelreste und die Grundsubstanz selbst sind Ebenso schützen basophile Schichten, wie die hypermineralisierten Grenzscheiden der Gefäße, die fälschlich als blaue Mäntel bezeichnet worden sind. Es sind dies nichts anderes, als basophile Halte- bzw Resorptionslinien. Der Stoffwechsel im Strähnenknochen ist nach all dem doch sehr vom lamellären Osteonknochen unterschieden, da foetale Merkmale wie hohe Osteocytendichte und hoher Gehalt an sauren MPS bis ins hohe Alter erhalten bleiben. Die Alterungsrate ist nieder Mikropetrosis ist selten echter Umbau findet nur wenig statt. Trotzdem findet ein ständiger Wechsel in den MPS und ihrem Polymerisationsgrad statt, nach Lipp (1955) stark energieverzehrende Prozesse.

Das in allen Details bereits von Meyer (1933) beschriebene Bauprinzip der menschlichen Labyrinthkapsel findet in den Beobachtungen Pedziwiatr (1967) eine interessante biomechanische Ergänzung die er in zwei Ver knöcherungssystemen sieht, der corona radiata labyrinthi und der intermediären Zone Dies unterstützt auch die Anordnung zweier Faser systeme der Strähnenknochen Äquivalent der intermediären Zone enthält ein sich mattenar tig verflechtendes Fasersystem, welches außer dem radiär und circulär ausgerichtet ist. Die Fasern der periostalen Kapsel hingegen sind in der Längsachse der Pyramide ausgerichtet. Unsere Untersuchungen zeigen weiters, warum ge rade di Labyrinthkapsel eine so große Fe stigkeit besitzt, wenngleich der Grad der Mine ralisation nach Engström & Röckert (1962) und Gussen (1967–68) nicht allzu hoch ist. Das Fasersystem und die Form des Füllstoffs vermitteln durch Elastizität einerseits und Quellbarkeit, sowie Viscosität andererseits Sta bilität. Gerade der hohe Gehalt der Grundsubstanz an sauren MPS entspricht durch Wasser bindungsfähigkeit und lockere Bindung an Kollagen gut diesen Voraussetzungen.

SUMMARY

By means of special decalcifying method and histological staining techniques we were able to study the rchitecture of the human labyrinthine capsule The

organic matrix contains dense network of canaliculi and lacunae and osteocytes in various functional stages. The ground substance consists f skein-like plaited fibr network and a cementing substance rich in acid mucopolysaccharides. Metabolism takes place as transformation f the mucopolysaccharides and a change in the grade of polymerisation. A remodelling process is shown as intralacunar resorption and apposition, morphological change and molecular transposition of the interglobular spaces and the formation of mantles along the vessels. The labyrinthine capsule is, according to our studies, a very active organ structure until advanced age. It long preserves its fetal signs and the maturing process is a very slow one. It is by no means an old bone or a dead chalk cliff.

RÉSUMÉ

On recherché la matrice d'os de la capsule du labyrinthe humain par une décalcification adéquate, par une technique histomorphologique et par une identification histochemique, et on constaté qu'elle est bien differente de l'os ostéonic lamellaire. Comme élément structurel on y trouve un os entrelacé, plein d'osteocytes et de canalicules. Le reste du cartilage est le centre de la modification. Dans la substance fondamentale on trouve une proportion riche de mucopolysaccharides acides. Cette modification de cellules se retrouve jusqu'à la vieillesse. Les résultats de cette modification sont des couches bleues mixtes et rouges.

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Remagen, W & Stolpmann, [illegible] Frage

Knochens nach Mjör (1962) und v d. Hooff (1964) darstellt und im wesentlichen nur ver kalken, aber nicht mineralisieren, das eine An nahme Gussens (1967–68) und die Untersu chungen Engströms & Röckerts (1962) bestätigen.

Wie an allen übrigen Knochen zeigt sich Stoffwechsel-Aktivität an den Osteocyten selbst, an ihrer Gestalt und der Dicke des MPS-Mantels z. B., aber auch an der Umge bung, welche zuerst Verlust der Basophilie und dann stark positive PAS-Reaktion zeigt. Dies heißt einerseits Wechsel im Polymerisationsgrad, aber auch Mengenzunahme der MPS nach Graumann (1958–64) Morphologisch äußert sich die Osteocyten Aktivität nach Lipp (1955) in intralacunärer Resorption, aber auch Apposition Dies kann — wenngleich am Strähnenknochen selten — zum völligen Zumauern der Lacunen führen. Es entsteht das Bild der Frost schen Mikropetrosis. Echten Umbau findet man am Strähnenknochen nur ausnahmsweise Lineare Apposition tritt nach dem zweiten Lebensjahr in den Hintergrund Knese (1963) sieht aber auch Zuwachs durch Reifung der Komponenten, d. h. Abnahme der IPS zu Gunsten des Kollagens und Zunahme Polymerisationsgrades und durch intraossale Neubildung. Die Flächendichte der Osteocyten nimmt ab und zwar nicht zuletzt durch Volumszunahme der Interzellulären Substanz. Dieser Alterungsprozeß verläuft am Strähnenknochen sehr langsam und dauert bis ins hohe Alter

Morphologisch faßbaren Umbau sahen wir an den interglobulären Räumen und zwar in derselben Form, wie Zawisch (1927) Lipp (1954) und Ruth (1961) wobei uns aber Zu sammenhänge mit den Manasse Weber schen Mantelbildungen aufgefallen sind Besonders in der Bogengangskapsel finden sich diese um ein Gefäß gelagerten Knochenneubildungen als blaue gemischte und rote Mäntel, je nach der Knochenart, die sie aufbauen Schutz vor regellosem Abbau bilden nach Lipp stark saure Areale wie es im Strähnenknochen die Knorpelreste und die Grundsubstanz selbst sind Ebenso schützen basophile Schichten, wie die hypermineralisierten Grenzscheiden der Gefäße die fälschlich als blaue Mäntel bezeichnet worden sind. Es sind dies nichts anderes, als basophile Halte bzw Resorptionslinien. Der Stoffwechsel im Strähnenknochen ist nach all dem doch sehr vom lamellären Osteonknochen unterschieden da foetale Merkmale, wie hohe Osteocytendichte und hoher Gehalt an sauren MPS bis ins hohe Alter erhalten bleiben. Die Alterungsrate ist nieder Mikropetrosis ist selten, echter Umbau findet nur wenig statt. Trotzdem findet ein ständiger Wechsel in den MPS und ihrem Polymerisationsgrad statt, nach Lipp (1955) stark energieverzehrende Prozesse.

Das in allen Details bereits von Meyer (1933) beschriebene Bauprinzip der menschlichen Labyrinthkapsel findet in den Beobachtungen Pedziwiatr (1967) eine interessante biomechanische Ergänzung, die er in zwei Ver knöcherungssystemen sieht, der corona radiata labyrinthi und der intermediären Zone Dies unterstützt auch die Anordnung zweier Faser systeme der Strähnenknochen, Äquivalent der intermediären Zone enthält ein sich mattenar tig verflechtendes Fasersystem, welches außer dem radiär und circulär ausgerichtet ist. Die Fasern der periostalen Kapsel hingegen sind in der Längsachse der Pyramide ausgerichtet. Unsere Untersuchungen zeigen weiters, warum ge rade di Labyrinthkapsel eine so große Fe stigkeit besitzt, wenngleich der Grad der Mine ralisation nach Engström & Röckert (1962) und Gussen (1967–68) nicht allzu hoch ist. Das Fasersystem und die Form des Füllstoffs vermitteln durch Elastizität einerseits und Quellbarkeit, sowie Viscosität andererseits Stabilität. Gerade der hohe Gehalt der Grundsubstanz an sauren MPS entspricht durch Wasser bindungsfähigkeit und lockere Bindung an Kollagen gut diesen Voraussetzungen.

SUMMARY

By means of special decalcifying methods and histological staini g techniques we were ble to study the rchitecture f the human labyrinthine capsule The

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THE ATTACHMENT OF THE CUPULAE, OTOLITH AND TECTORIAL MEMBRANES TO THE SENSORY CELL AREAS

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Abstract Using colloidal iron staining or post fixation with ruthenium tetroxide it could be evidenced that the interstitial cells on the sensory areas in the inner ear in pigeons produce a fibrillar network surrounding every hair bundle of the sensory cells. This network ties the overlying membrane to the surface of the sensory area. The compartments created by the interstitial cells constitute fluid-filled plastic tubes in which the hairs are suspended. This arrangement gives mechanically a preference for shearing movement, which bends the hair bundles in their compartments in exact accordance with the fluid displacement in the canal. This gives the highest sensitivity with the lowest expenditure of energy Morphological reasons for continuous renewal and a slow disaggregation of the cupulae, tectorial and other membranes are presented.

The problem of the attachment of what has been called the gelatinous membranes" to the surface of the sensory cell areas in the inner ear is an old problem which does not seem to have been satisfactorily solved. Even if the investigations into the relation between specially the tectorial membrane and organ of Corti has pointed towards a relatively firm attachment to the Hensen cells when observed in the living animal (Hilding, 1952 v Békésy 1953) there is still confusion with regard to the mechanism by which the hairs of the sensory cells are bent by the shearing movements between the hair cells and *inter alia* the tectorial membrane. There have been several observations reported of an occasional contact of a few hairs with the filaments of the tectorial membrane as seen in the electron microscope (Iurato 1960) Kimura (1966) has in an extensive study of this problem on several species of mammals been able to show that the longest of the hairs on the outer hair cells touch or are embedded in the ground substance of the tectorial membrane for a short distance. The hairs of the inner hair cells, however were free floating in the subtectorial space and showed no connections with the tectorial membrane. Most investigators are, however quite convinced that there is no structural continuity of the hairs with the tectorial membrane (Smith & Dempsey 1957 Spoendlin, 1957 Engström & Wersäll, 1958 Iurato 1961)

When the cupula was experimentally removed in pigeons (Dohlman & Boord, 1964) it could be shown in light microscopy that the hairs of the sensory cells within a few hours after the operation apparently were unaffected and were bent in all different directions as would be expected from unsupported and flexible hair bundles in a fluid like the endolymph. This contradicts any assumption of a firm attachment to the cupular ground substance and raised the question how the hairs on the cristae are held in position by the cupular structures in the living animal. It is well known that the hair cells respond in a closely correlated manner to the degree of physiological stimulus to which they are exposed (C ., 195 Ledoux, 1958 and ot) uisite

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such a coordination of stimulation and response requires the closest correlation between the mechanical events at every step of the stimulatory process in the semicircular canals and their ampulla and specifically between the displacement of the cupula and the corresponding bending of the hairs, if this is the mechanism by which hair cell stimulation is accomplished as is generally assumed.

In light microscopy of the cristae or otolithic membranes there is always a translucent area of varying width between the surface of the hair cells and the structure of the cupula or otolith membrane This has been called the subcupular space" constituting a slit through which the hairs are seen to pass into the organized canal-like structures of the cupula. This arrangement gives the impression that there could be some support to the hairs above the subcupular space. These structures, the cupula and probably also the subcupular space shrink considerably when subjected to fixations and dehydrating procedures. It is therefore impossible to assess the proper relationship between the hairs and the structure of the cupulae or other similar membrane from the histological picture of these membranes. There is, however o indication that the cupula is tied to the ista, when examined in well fixed specimens d when the plane of sectioning is correctly chosen. This leaves the impression of a cupula free floating over the surface of the crista separated from the surface of the hair cells area by the empty slit of the subcupular space. To study the sub-cupular space and structures which logically may exist to tie the cupula and similar membranes to the surface of the sensory area and to visualize the place of the hair bundles in this environment has been the purpose of this investigation. Two methods have been used for an electron microscopic investigation of these areas in the pigeon s inner ear the colloidal iron method by Hale (1946) as modified by Rinehart & Abul Haj (1951) to stain acid mucopolysaccharides and the fixation with ruthenium tetroxide by Gaylarde & Sarkany (1968)

METHODS

Colloidal iron (Hale, 1946 Rinehart & Abul-Haj, 1951)

The bony ampullary walls were opened with $^1/_2$ mm dental drill and a pointed scalpel N 11 The bone over the vestibulum was removed in the same way In the living or just decapitated pigeon the ampullae were fixed by flushing the labyrinth with 3–6.25% glutaraldehyde in cacodylate buffer at pH 7 4 After 2–3 min all the canals were sectioned close to the ampullae the lateral corner of the utricular macula was grasped with fine watchmakers tweezers and the horizontal and anterior vertical ampullae could in this way be removed. After sectioning the nerve, the posterior vertical ampulla could also easily be lifted out for continued fixation in glutaraldehyde for 2–4 hours. Washed in 0.25 M sucrose Stained in dialyzed colloidal iron at pH 1 8 for 6 hours. Then washed in distilled water for 15 min. Dehydrated in ethanol and embedded in Araldite

Fixation with ruthenium tetroxide (Gaylarde & Sakarny 1968)

Glutaraldehyde fixation 3% overnight (cacodylate buffer pH 7 4) Washed in buffer 4 × 30 min. Postfixation in 0 1% ruthenium tetroxide in sodium acetate buffer pH 7 0 for 1 hour at 4 C Washed in buffer dehydrated in ethanol. Embedded in Araldite or in Spurrs low viscosity embedding medium

RESULTS

The most striking observation with regard to the subcupular space when using these methods is that this space" is not as empty as it appears in light or electron microscopy when conventional methods are used This is most evident in the cochlea of the pigeon where the tectorial membrane is more solid than in mammals and therefore does not shrink to the same extent. In cross sections through the basilar membrane with its hair cells (the birds

Fig. 1 Light microscopy cross section of the cochlea. Tectorial membrane (*TM*). Tegmentum vasculosum (*TV*) with dark and light cells. "Long slender cells (*SL*). Hair cells (*HC*) colloidal iron stain.

have no organ of Corti, only a layer of hair cells over the whole basilar membrane) and the tectorial membrane as illustrated in Fig. 1 the results of staining with colloidal iron is shown. In this 1 μ thick section examined in light microscopy the dense structure of the tectorial membrane containing holes and empty spaces are demonstrated. In the subtectorial space" the hair bundles are seen passing through this space from the surface of the hair





Fig. 2 (*a*). Light microscopy higher magnification showing hair cells (*HC*) with their hair bundles (*H*) and supporting cells (*SC*) with their microvilli and bands (*V*) connecting the microvilli with the tectorial membrane (*TM*). (*b*) The same area in electroscopy in about 3 higher magnification.

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Fig 4 Section parallel to the surface of the hair cells. Hair cells (*HC*) with cross section of the hair bundles of 100–120 stereocilia and one kinocilium (*KC*). Each hair cell is surrounded by the supporting cells with their microvilli (*MV*). The hair bundles are encased in a tube-like compartment produced by the "cells" (*V*) from the microvilli to the tectorial membrane.

microvilli are dense but without a clearly defined outer membrane. The surface shows a direct continuation with the fibrils giving the impression that the fibrils are split off or shed by the microvilli. From these structures the fibrils are arranged in a loose cotton-like fabric mostly in parallel stretches continuing to the fingerlike extentions of the tectorial membrane. It also seems evident that the fibrils of the bands are applied to the surface of these extentions, loosely in the periphery and more firmly packed when they seem to be attached to and creating the ground substance of the tectorial membrane.

The morphology of the interstitial cells in electron microscopy has earlier been described in a variety of animal species (Smith, 1967 Wersäll, 1956 Fleck, 1965 Spoendlin 1957) They seem all to be very similar showing only minor variations They extend from the basal lamina to the endolymphatic surface. They usually carry several microvilli on this surface.

In the pigeon these cells have developed a thick brim of microvilli. The nucleus is irregular in form, situated in the lower part of the cells. The cytoplasm contains some relatively small mitochondria, few rough surfaced endoplasmic reticula and a medium-sized Golgi complex, all of these organelles are located in the thin part of the cell not too far from the endolymphatic surface In the region closest to the microvilli the cytoplasm is of higher electron density which gives the impression to be due to an increasing density of fine fibrils and tubules.

The "long slender cells" (Fig. 6) in the upper frontal wall of the cochlear duct are forming a firm support for the broadest and most compact part of the tectorial membrane They take part in the creation of this membrane in



Fig 5 Hair cells (*HC*) with lengthwise sectioned hair bundles (*H*). Between the hair bundles are sectioned microvilli (*MV*) from the supporting cells From the microvilli is seen the loose fabric of fibrils constituting the veils encasing the hair bundles. Glutaraldehyde, ruthenium tetroxide

embryonic life and are apparently functionally and morphologically equivalent to the inter cells on the limbus of the mammalian cochlea. These long cells in the pigeon cochlea have mostly a light cytoplasm, a rounded or oval nucleus at varying height, small Golgi complexes and few rough endoplasmic reticula and relatively few mitochondria. Specific for these cells is the type of lysosomes, membrane bounded inclusion bodies with electron dense granula and often one to several lipid droplets, sometimes also containing remnants of membranes. Beside the aggregations of dark granules in the lysosomes there are dark inclusion bodies of varying sizes in the cytoplasm. There are also several pedunculated vesicles" from the cell membrane extending into the tectorial membrane Further they might sometimes show a large cluster of smooth endoplasmic reticula Both these types of organelles are also found in the light cells of tegmentum vasculosum, the vascularized ridges hanging down from the ceiling of the cochlear duct over the whole tectorial membrane The lysosomes in both types of cells have an electron microscopic appearance which is very much the same It was shown in an earlier investigation (Dohlman, in prep) that the light cells in tegmentum vasculosum sometimes expel these organelles into the endolymph where they can be found on the surface of the tectorial membrane It was also shown that the same type of lysosomes either as whole lysosomal bodies or as smaller electron dense granula passed from the long cells into the ground substance of the tectorial membrane (Fig. 7) In both cases the lysosomes seemed to have an enzymatic decomposing or disaggregating effect on the ground substance of the tectorial membrane manifesting itself by producing holes in the membrane where the lysosomes are expelled from the long cells and lacerating the upper



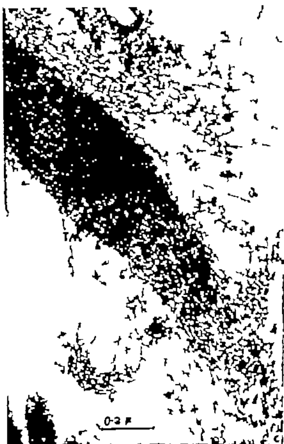


Fig 5 c. The fibrils from the veils (*V*) are seen to attach themselves to the extension of the tectorial membrane (*TM*). Glutaraldehyde, ruthenium [illegible] ide.

surface of the tectorial membrane [illegible] when the lysosomes from tegment[illegible] sum fell down on that surface [illegible] in prep.) Accordingly all the holes [illegible] membrane contain small electron[illegible] which seem to be melting [illegible] ing fibrils. However this [illegible] be specific for the pigeon ear [illegible] all published electron [illegible] membranes also from [illegible]

Fig 5 b Section of a [illegible] the supporting cell [illegible] From the surface of [illegible] brils of the veils [illegible] mem[illegible] f the tectorial [illegible] and kinocilium [illegible]



Fig 6. Two slender cell (*SL*) and the base of the tectorial membrane (*TM*). The cells show protrusions into the tectorial membrane (*P*). They contain dark inclusion bodies (*DB*) which also are found in the tectorial membrane usually i connection with holes in its substance

does not seem to have been mentioned or ex

DISCUSSION

The general structure of the cupulae otolith membranes and the tectorial membrane in mammals as seen in light microscopy is well known from the description in every textbook and paper dealing with different aspects of these structures. The interpretations of the true nature of these membranes have varied due to the image these structures present when subjected to different methods of fixation and staining. A more clear concept of the structure constituents and most important the relationship of these membranes to the sensory areas, cannot be expected to be gained without the greater possibilities offered by electron microscopic investigations with different auxiliary techniques.

In 1956 Wersall could evidence a reticulum of fibrils of about 100 A in diameter as the basic structure of the cupular substance Spoendlin (1957), Engström & Wersäll (1958) Iurato (1960) found the tectorial membrane in guinea pigs and rats to be formed of extremely thin fibrils of about 40 A (Engström & Wersäll, 1958) to 100 A (Iurato, 1960) or being of irregular thickness (Spoendlin 1957) From this it is evident that there is general agreement with regard to the nature of the ground substance of these so called gelatinous membranes" They are composed of tightly packed fibrils of a uniform character They might vary to some extent in length and thickness, but always retaining the fibrillar structure. These gelatinous membranes" have therefore nothing in common with amorphous gelatinous structures. The chemical analysis of cupulae and tectorial membranes by Iurato showed that they contained a protein belonging to the keratin group. This is what could be

expected when produced by epithelia of epider mal origin as those covering the inner walls of the membranous inner ear

It is further generally agreed that the bending of the hairs of the sensory cells is the essential link for transmitting the mechanical energy from the cupulae, otolith membranes or the tectorial membrane to the sensory cell for its excitation. This is the main problem dealt with in this paper The mechanism by which the bending of the hairs stimulates and inhibits the actions of the hair cells on the nerve endings has been a matter of discussion for a long time still based on insufficient factual data for a solution, and will not be considered in this connection.

The ground substance of the cupulae produces an image of longitudinal canals and in the mammalian tectorial membrane, radial as well as longitudinal fibres. This image can change to some degree with different methods of fixation as pointed out by Spoendlin (1957) but in the cupulae the general orientation of the canal like meshwork is always the same (Fig. 9 *a* and *b* 10 11) In preparations of the ampullae stained with colloidal iron the sponge like meshwork of the cupulae is well illustrat ed. It shows the ground substance as canal walls in cross section (Fig. 10) and longitudinal beams when sectioned lengthwise (Fig. 11) This ground substance may be more or less condensed and shrunken by the fixational and dehydrating procedures used, but they are always present in the same general arrange ment. The tectorial membrane, in the pigeon, seems to be more stable and less affected by fixation and dehydration probably due to the greater density of the bulk of its ground substance. At the surface of the tectorial membrane a thin precipitation of stained mucopoly saccharides can be demonstrated (Fig. 3) whereas the surface of the cupular trabeculae always are heavily stained (Figs. 10 11). Much of the endolymphatic fluid within the meshwork of the cupula is also stained. In the tectorial membrane, on the other hand, the holes seen in the compact ground substance are usually without colloidal iron stain, indicating that these holes do not generally communicate with the endolymph. It therefore seems more likely that they might be the result of an "inside digestive process created by enzymes from lysosomes expelled from the "long slender cells or the light cells in tegmentum vasculosum as discussed in other connections (Dohlman, in prep) The difference in density of the colloidal iron staining, strong in the ampulla, weak in the cochlea reflects the lack of mucopolysaccharide secreting cells in the cochlea on one hand and the presence of the large polysaccharide secreting planum semilunatum areas in the ampullae on the other This difference in mucopolysaccharide content in the two parts, the cochlea and the ampulla respectively seems to imply that the mucopolysaccharides in the endolymph cannot be essential for the



Fig 7 In most of the holes in the tectorial membrane, small grainlike dots are found, assumed to be residues of lysosomes containing Keratolytic enzymes, creating the holes

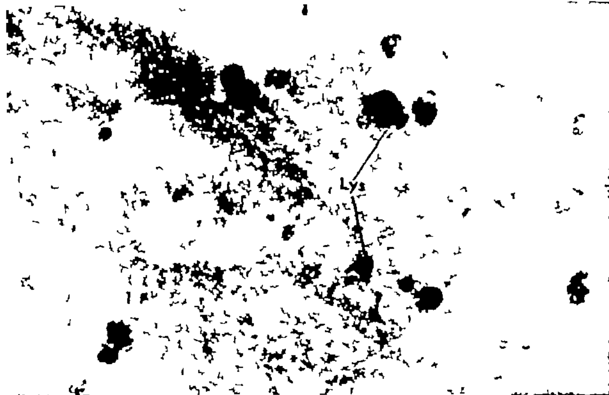


Fig 8 Lysosomes (*Ly*) of the same type as those found in the light cells of tegmentum vasculosum and in the slender cells are seen in the holes and on the lacerated surface of the tectorial membrane.

process of hair cell stimulation per se, but be of importance only for the mechanical transmission of inertial endolymph movements to the cupula in the canal-ampulla complex (Dohlman, in prep) It has been shown by Hardesty and v d. Stricht that the embryonal tectorial membrane developes from the cells on the anterior upper wall of the cochlear duct, the cells which develop to the long slender cells" and from those which differentiate to the sensory cell area on the basilar membrane. It may however be assumed that no structure in the body remains unchangingly the same all through life That must also apply to the membranous structures over the sensory areas. This has been proven by removal of the cupula in pigeons and studying the reformation of this structure with the return of canal function (Dohlman & Boord, 1964 Dohlman, in prep). In the adult animal it might therefore be assumed that the renewal of these membranes may originate from the cells which created them in early embryonic life From this point of view the interstitial cells on the cristae and the basilar membrane of the cochlea, as well as the "long slender cells" can be assumed to be the cells producing the building stones for the ground substance of the cupulae and the tectorial membrane respectively

The specimen stained with colloidal iron showed a light staining of the "veils" from the microvilli of the interstitial cells to the tectorial membrane. It cannot be decided from the appearance of the fibrils in the "veils" if the fibrils themselves are stained in which case they should be composed of mucopolysaccharides, or if they only are covered by a faint layer of saccharides stained covering a fibrillar ground substance of proteins.

When investigated with ruthenium tetroxide fixation these "veils showed the network of extremely fine fibrils in direct continuation with the fibrils of the tectorial membrane on one hand and the microvilli on the

Fig 9 a. Section through the ampulla. Planum semilunatum and hair cells with their hair bundles. "Veils" between the hair bundles (*V*). Mucopolysaccharides stained in the endolymph (*MPS*). Cupula (*C*) colloidal iron.

other This could be interpreted as indicating a process of production by the interstitial cells which in producing the fibrils were contributing to a slow process of formation of the ground substance of the tectorial membrane. The general occurrence of relatively long finger like extensions of the tectorial membrane meeting each section of the "veil" appears to fa-



Fig 9 b Light microscopy section of an upper part of cupula in cat.

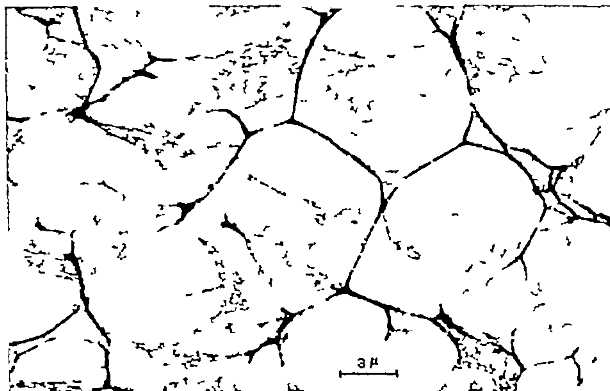


Fig 10 Cross section of the canals of the cupula. Within the canals are mucopolysaccharides stained with colloidal iron.

vour a concept of an application of fibrils to the structure of the membranous ground substance.

The fibrils at the level of the microvilli of the interstitial cells are seen to be partly attached to the surface of these microvilli and are, when sectioned lengthwise, giving the impression of being split off from this surface. This has been interpreted as a process of shedding the fibrils from the surface of the dense and homogeneous microvilli which do not seem to have any cell membrane. The ruthenium tetroxide is an exceedingly good fixation for cell membranes as evidenced for instance in Fig. 5 *a* at the tight junctions between the hair cells and the interstitial cells. If the microvilli should have a limiting membrane in its proper sense this should be expected to be visualized with this fixation.

From the lengthwise arrangement of the fibrils in the "bands" to the tectorial membrane extension it might be inferred that they are being subjected to stretch. The continuous exposure to sound producing an up and down movement of the basilar membrane may thus be the basis for the stretching of these bands. It is therefore assumed that a continuous shedding of new fibrils, stretched and relaxed by the sound movements are attached to and packed together at the surface of the existing tectorial membrane. Application of new fibrils may thus contribute to the growing of the tectorial membrane as the finger like extensions indicate, everywhere in direct connection with the "bands" in the two-dimensional reproduction or as "veils" in a three-dimensional view. This explanation of a growing tectorial membrane is conjectural in need of further supporting evidence and is therefore mentioned here as a working hypothesis but is supported by the evidence of the new formation of the cupula after experimental removal.

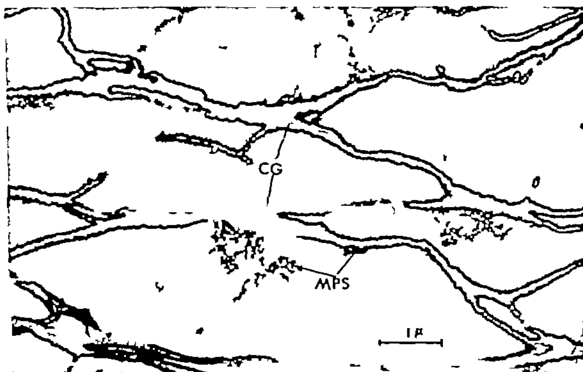


Fig. 11. Lengthwise section of the cupula in higher magnification. The ground substance of the cupulas meshwork (*CG*) is covered by colloidal iron-stained mucopolysaccharides (*MPS*).

It is obvious from the image of the bent hairs in their compartments (Fig. 3) that the "veils" are elastic enough to be stretched probably due to the loose fabric of their fibrils, but also sufficiently tenacious to tie the membrane to the sensory area. This implies that the "subcupular space" is not the empty space it has been believed to be. It contains all the elastic "veils" around every hair cell of the cristae. Together some 20–30 000 of them, they provide a firm anchorage of the cupula. The only freedom of movement which this arrangement allows the cupular structure is that of sliding. Such a movement, within certain limits over the sensory area meets only an insignificant resistance and can therefore be performed with the lowest energy expenditure compared with other ways of a mechanical angular displacement of the hair bundles. In a shearing movement of the cupula, the compartments encasing the hair bundles are bent as a unit. Within this fluid-filled compartment the hair bundle follows the swaying of the enclosed fluid without the need for any other fixation to the sliding cupula or tectorial membrane.

The bending of the cupula as a whole has experimentally been shown to produce a stimulation of the sensory epithelium and reactions from the effector organs (Steinhausen, Dohlman Trincker 1959 Ledoux, 1958) Experiments aimed at correlating the bending of the cupula and the response as action potential frequency in the ampullary nerve have, however failed due to the extreme sensitivity of the hair cells to fluid movements in the canal (Dohlman, in prep.) The response seems to have reached near maximum values already before a cupular deflection could be recorded. This seems to favour a mechanism of stimulation, other than bending of the cupula as a whole, for stimulation of the sensory cells at values within the physiologic range. Therefore

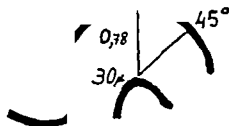


Fig 12 Explanation in the text.

the importance of the shearing movements of the cupula must be emphasized also from this point of view

Fig. 12 is designed to illustrate the difference between the concept of bending the hairs due to deviation of the cupula as a whole, and that of the hair cell stimulation by means of a shearing displacement.

Assuming on one hand a deviation of 45 of the hair bundles performed exclusively by the rotation of the cupula, the volume of displaced fluid in the canal system is $\pi r^2/8 \times Y$ where r is the distance from the crista to the vertex and Y the surface of the cupula. On the other hand the same 45 deviation of the same hair bundle can be presumed to be produced by a purely shearing movement of the cupula sliding over the surface of the crista. In this case a displacement of the cupula is performed of only $l \sqrt{2} \times r \times Y$ where l is the length of the hair bent in the subcupular space If r is estimated to be 0 78 mm and l 30 μ the relationship between these two volumes will amount to be about 20 times greater in case of a deviation of the whole cupula than with a shearing movement. This is only mentioned to demonstrate the small endolymph volume needed and the high sensitivity resulting from a sliding movement compared to the bending of the whole cupula. In reality these two functional mechanisms, shearing and bending, must be assumed to act simultaneously The shearing movement can, however produce a deviation of the hair bundles earlier and to a great extent explain the extremely high sensitivity of the canal system to angular accelerations, whereas the deviation of the whole cupula takes up a large volume of displaced endolymph, thereby preventing damage to the hair cell region in cases of supernormal stimulation.

A deflection of the entire cupula without a shearing movement would further require that the cupula is firmly tied to the side walls of the crista with some elastic reinforcements. Morphologically such structures have not been found The cupula is separated from the crista everywhere by the "subcupular space" The presence of the "veils" therefore clearly facilitates a sliding displacement over the hair cell area, underlining the importance of the concept of a shearing movement as requiring the lowest volume displacement of the endolymph in the canals consequently giving the highest sensitivity with the lowest energy expenditure

To this can be added the presence of mucopolysaccharides in the endolymph of the canal system earlier mentioned. The finding of a gel-forming compound in the endolymph in negative staining" (Dohlman & Pinteric, in prep.) and a viscosity roughly twice that of water (Money et al., 1971) indicates that the cupula is surrounded by a fluid which is restricting the movements of the cupula and thereby facilitates a shearing movement over the crista (Dohlman, in prep).

The observation of hair tips attached to or even embedded in the tectorial membrane which was so well illustrated by Kimura has been confirmed in this study If this should have any meaningful physiologic implication it would be necessary to assume that in the living animal not only single hairs should have this attachment but all the hairs in the hair bundle. Kimura (1966) discussed this problem but could not add any proof for this hypothesis. However assuming a physiologic attachment of some or several hair tips, it appears to be a dangerous arrangement to expose only the uttermost tips of a fragile and presumably not elastically stretchable hair to vibrations of 10 or perhaps 20 000 cycles per second when the

rest of the hairs are floating in a free fluid space Therefore, I am convinced that this cannot be the physiologic mechanism for bending the hair bundles.

The arrangement of the hair bundles enclosed in the elastic compartments offers a quite different stability to the system. Each hair bundle is suspended in what can be described as a short elastic tube filled with fluid. When the tube, i.e the walls of the "veils is bent, *the fluid content in the tube and consequently* also the whole hair bundle in this tube is bent. This is from a physical point of view the most gentle way in which such a movement can be transferred to the hair bundle and it has, further the great advantage that it produces an exact correlation between the shearing movement and the resulting bending of the hair bundle. This is the most important consequence of the concept of compartmentalization of the hair bundles. It provides the necessary basis for this exact correlation which could not be achieved by the assumption of free floating hairs even if some of the longest hairs have a support at their extreme end while others, such as the hairs of the inner hair cells, should have no connection with the surface of the tectorial membrane

Further the fine woven fabric of the "veils" extending from the surface of the thin extentions of the cell body of the "interstial cells *embracing the hair cells, are of interest from* the point of view of a growing tectorial membrane. There does not seem to be any difference between the fibers constituting the tectorial membrane and those of the "veils There are all stages of transition from the loose fabric of the "veils and the tightly packed fibres of the ground substance of the tectorial membrane. The finger-like extentions of the tectorial membrane, meeting the "veils seems to indicate that they are produced by the continuous application of fibres from the "veils" as building stones for the ground substance of the membrane.

The second source of renewal of the tectorial membrane seems to be the "long slender cells" They also contain fibrils resembling the fibers of the membrane attached to their surface. There is, however no proof that the fibres seen in the cytoplasm of these cells are exported in the ready-made fashion in which they appear in the cell. They could represent some precursor substance precipitated by the fixation processes. This problem is the same as that of collagen production by the fibroblasts.

Further the process of production of fibrils *which build the ground substance of the tec*torial and other vestibular membranes by application and tight packing of the fibrils supplied by the interstitial cells, is balanced by the action of lysosomes lacerating the surface of the tectorial membrane and melting the ground substance from within by creating holes in the membrane.

Most conspicuous is the sequence of fibrils and tubules, found first within the cytoplasm of the supporting cells as well as the "long slender cells" then the fibrils shed by the microvilli, and from there as a loose cottony veil to the felt-like structure of the cupula or tectorial membrane. This production of "veils by the interstitial cells creates "tubes" due to the fact that the interstitial cells totally surround each single hair cell. It is therefore assumed that the growth of these tubes from the interstitial cells results in the canals which are the significant structure building the cu*pulae. In the cochlea where the finger-like ex*tentions of the tectorial membrane meet the "veils the same process seems to start building up similar canals. The cochlear partition is, however continuously subjected to the vibrations by sound, contrary to the slow movements in the canal system. These vibrations could, thus, be assumed to pack the fibrils in a different pattern producing the difference in structure of these two membranes.

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Toronto and with much valuable assistance from the Defence Research Establishment, Toronto, as well as the Medical Research Council of Canada.

ZUSAMMENFASSUNG

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DISCUSSION

S. Iurato: What are the technical details of the operation for cupula removal and did Mr Dohlman follow the process of producing new cupula

J Wersäll. Today we have unique occasion with so many specialists in inner ear morphology and physiology I would like to take the opportunity to ask with reference to the close attachment of the tectorial membrane to the supporting cells whether travelling wave exists in the cochlea of the bird as in the guinea pig. If it exists, how and in which direction is the energy transformed upon the hairs of the sensory cell?

Cath. Smith: W (Takasako & Smith) have been studying the pigeon's cochlea also and can confirm M Dohlman findings that fibers from the tectorial membrane appear to be attached to the microvilli of the supporting cells. It was not clear to me whether the speaker meant that all the hairs were in contact with the tectorial membrane. In our material this was not so. Only the longest hairs, which include the kinocilium on the pigeon cochlear hair cells, reach the tectorial membrane. The shorter hairs (about half of those in the bundle) do not touch the tectorial membrane. This raises the question of whether some movements of the basilar membrane move all the hairs, whereas others move only the longest ones.

J Tonndorf I do not share the reservation of M Dohlman concerning the possibility of injury of the hair tectorial membrane junction. The 1 4 wave length at, say 10 kHz, is still more than 3 orders of magnitude larger than the size of the hair cells. However I would be afraid that in the manner the speaker suggested (hydraulic) cancellations might occur I would like to suggest the following mechanism in accord with Békésy observations: The long hairs are in contact with the tectorial membrane and are being translated in the shearing motion. The short hairs serve to back up fractional resonances of the long hairs. Travelling waves, in answer to M Wersäll question, most likely occur. They do so in the amphibian ear (van Bergeijk). I would be more than surprised if they did not occur as their occurrence is hard to avoid even when one tries.

G Kearney According to our concept regarding rubella deafness, the origin in destruction of the cupula by hemorrhage (red corpuscles figure as specific poison to the cupular substance). Question: does the cupula regenerate?

G Dohlman (Reply) to M I rato. You asked about the technique used to remove the cupula. This is not a difficult operation. A small piece of the bony ampullary wall is lifted off to get access to the membranous wall. This wall is incised with sharp pointed needle or knife. A fine glass capillary of 0.05–0.1 mm bore is used as suction tube, introduced into the ampulla through the hole and when suction is then carefully applied, the membranous walls collapse when the endolymph with the cupula are thus removed. Microscopic sections of the crista few hours after this operation show the hairs on the hair cells to float in all directions as if they were without any support. Later they regain their regular position and in the picture I showed from pigeon operated on more than a year before sacrificing, functionally normal and morphologically restored cupula had been found, practically identical in the ampullae of both sides.

To Mr *Wersäll*. As my main interest has always been centered on the vestibular part of the ear I don't feel competent to answer your question about the travelling waves with regard to structures here described. However as far as I can see, the response to different wa forms cannot be changed or affected by the structural arrangement around the hair bundles. Only the correlation between stimulation and response must increase in sensitivity and exactness.

To Miss *Smith*. You mentioned two morphological facts with regard to the relation between the hair cells and the tectorial membrane. The first was that only the long hairs might reach the tectorial membrane, and second, that some hair brushes might be excentrically placed on the hair cell. I believe that the ell" from the interstitial cells to the tectoral membrane, enclosing each of the hair brushes of the sensory cells in its special compartment, is the important factor transmitting the stimulating movements to the hair cell and that w may therefore, disregard the occasional connections of the hairs to the membrane, or their place in this compartment.

To Mr *Tonndorf*: I may be wrong in assuming damaging effect on the embedded tips of the hairs. However the importance in this concept of enclosing the hairs in their compartments of the keratin fabric is the difference between free floatin hairs they can be seen after removal of the cupula in an inability to react, and on the other correlation between endolymph mov the modulation of action nal conditions. This ted only by the touching of f few hairs. I still bel comb-arrangement, ally the important formation of the ing of the hair tuf hairs are bent in hal tufts are bent as problem ha on these m

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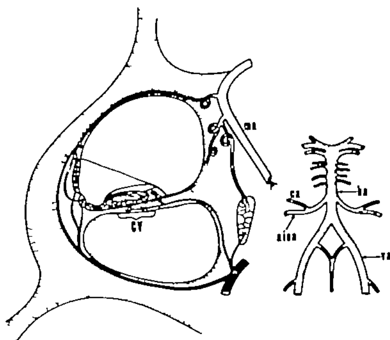


Fig 1 Schematic drawing of the blood supply to the scala media. The area marked *CV* (Ciné view) indicates the region of view for the motion picture camera in the basal region. *ma*, modiolar arteriole; *ca*, cochlear artery; *ba*, basilar artery; *aica* anterior inferior cerebellar artery; *va*, vertebral artery

rence, 1966) demonstrated that when the arterioles of the modiolus radiating towards the spiral osseous lamina and basilar membrane are occluded, the organ of Corti in the region of occlusion degenerates while the stria vascularis remains normal. On the other hand, when the vessels radiating out over the scala vestibuli and extending down to the spiral ligament and stria vascularis are occluded, those structures degenerate, but the organ of Corti remains histologically normal.

As interest in the role of these spiral vessels has increased, more evidence indicating their important role in the function of the organ of Corti has been accumulating. Nakai & Hilding (1967) found that the highest activity of adenosine triphosphatase is found in the basement membrane surrounding these vessels. Hawkins (1967) reporting at the Third Symposium on the Role of Vestibular Organs in Space Exploration, showed pictures from his surface preparations of the guinea pig, unmyelinated nerve fibers running to the inner spiral vessels with nerve endings en passant.

Pathological changes have also been demonstrated in these vessels associated with factors known to produce temporary hearing losses. Lawrence et al. (1967) reported an absence of erythrocytes in both the stria vascularis and in the spiral vessels associated with small shifts in electrical activity (decrement) following relatively moderate sound levels of stimulation in the guinea pig. Hawkins (1967) has reported that for adequate dosages of both quinine (quinine dihydrochloride) and salicylates (sodium salicylate) there is partial occlusion of the spiral capillary vessels by swollen endothelial cells which block the passage of red blood cells. This, of course is a most important observation, when coupled with the fact that occlusion of these vessels over an extended period causes a degeneration of the organ of Corti.

In an effort to observe the occlusion of these vessels by swollen endothelial cells following drug administration we have developed a method of observing the flow of blood through the capillaries visually and photographically through the microscope.

During the course of exploring the organ of Corti with a microelectrode to determine the distribution of resting potentials (Lawrence & Nuttall, 1970) it was noted that the spiral ves-

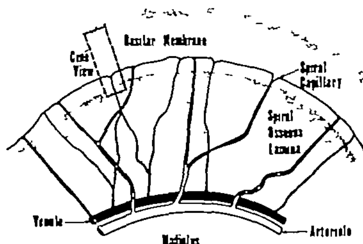


Fig Schematic view of the spiral osseous lamina and basilar membrane to show the distribution of spiral capillaries on the basilar membrane. For simplicity the sporadically appearing spiral vessels of the tympanic lip on the spiral osseous lamina have been eliminated. The rectangle indicated ciné view outlines a "T" junction and represents the field of view seen in the motion pictures. The organ of Corti lies on the other side of the basilar membrane

sels of the basilar membrane and of the spiral osseous lamina could readily be seen. With proper surgical approach and illumination, it is possible to see up through the organ of Corti, to look up into the tunnel space, to see the feet of the pillar cells, the rods of Deiters cells, the striations of the basilar membrane and Claudius cells. Subsequently we launched a program to investigate the flow of blood through these capillaries under normal conditions and under the influence of drugs known produce a temporary threshold shift.

This report is mainly to describe the technique and present the motion pictures made with the high-speed motion picture camera. The results of these observations have been described earlier following injection of quinine dihydrochloride (Lawrence, 1970)

METHODS

At the Kresge Hearing Research Institute we have a colony of guinea pigs which are bred specifically for our experimental purposes in order to minimize the incidence of middle ear infection and to provide constancy in reaction to anesthesia and drugs. The animals used for the purpose of making these motion pictures average 450 g at the time of operation, and are anesthetized with Dial [1] no curare is used In these animals because this is known to release histamine which not only produces a certain amount of pulmonary edema in the guinea pig, but might have an unpredicted reaction upon the terminal capillaries.

The bulla is exposed by a ventral approach and the tympanic membrane and annulus along with the ossicular chain are removed. After the performance of a stapedectomy a small wedge of bone is taken from the edge of the round window and the round window membrane removed in order to expose the basilar membrane. When the basilar membrane is so exposed, the structures within the scala media can be seen and the spiral vessels on the basilar membrane are readily observed.

Fig. 2 shows the exposure as seen under the low power microscope (Lawrence et al., 1967) of the spiral osseous lamina, basilar membrane, and bony ledge beneath the spiral ligament. This bony ledge beneath the spiral ligament is a characteristic structure of the basal turn in the guinea pig. The high-power microscope and motion picture camera see only the region outlined as the ciné view" indicated by a rectangle

Dial [1] and Urethane solution. Ciba Pharmaceutical Co., Summit, New Jersey

The animal is mounted with its head anchored in a special headholder which is clamped to the tabletop This clamp grabs the zygomatic processes and completely immobilizes the animal's head.

Two microscopes are used, one is a binocular microscope used for operating and has a maximum magnification of about ×65 This microscope is mounted on an arm which also is anchored to the tabletop so that the microscope can be swung out of the way Another monocular microscope also mounted on an arm can be swung into position directly over the animal's head and the basilar membrane This microscope has a ×10 occular and the objectives can be changed to any particular one desired. The occulars used are the Leitz Wetzlar UM series with a long working distance. These are extremely fine objectives and contain an iris diaphragm.

Mounted on a steel rod suspended from the ceiling is a counterweighted Mitchell 16 professional camera. This camera can be swung into position directly over the monocular microscope and the animal. Various intermittent framing rates are possible and in this particular motion picture, framing rates up to 128 frames per second have been employed.

Two light sources have been used. The first was a fiberoptics light source which gave sufficient light for the use of a ×32 objective and a framing rate of 32 frames per second. However this light, although cool, is quite yellow and lacks intensity for the higher framing rates. Another light source, the Chadwick-Helmuth point source strobex, has the capability of being triggered by the camera frame-drive motor so that the strobe flashes at the exposure of each frame For the speeds of 0–64 frames per second, the flash duration is 40 μsec at 128 frames per second, the flash duration is 25 μsec. This light source has good color characteristics and because of the short duration of the flash does not overheat the tissue.

During the photography of the blood flow in the spiral vessels, the pulse rate by means of an electrocardiograph and the blood pressure are also recorded. The electrocardiograph is recorded by means of electrodes placed on the chest wall leading through an amplifier to a channel of a Grass Recorder This is also monitored on a cathode-ray oscilloscope so that the condition of the animal can be determined continuously The left carotid artery is cannulated for blood pressure measurements which are read on another channel of the Grass Recorder

The animal is artificially respirated with a rate and air volume that maintains the animal in a normal physiological condition as determined by his ECG and blood pressure over long periods of time.

When the motion pictures are taken, the perilymph resting over the basilar membrane is absorbed with a pledget of cotton and replaced by Dow Corning medical fluid 360 heated to a temperature of 37 C. This not only keeps the basilar membrane and blood vessels from drying out but seems to have optical qualities that allow a good view of the structures of interest A pledget of cotton is also placed within the oval window to absorb the perilymph and Dow Corning fluid so as to permit the reflection of light up through the organ of Corti from the walls of the vestibule When this is successfully accomplished not only can the blood flow through the capillaries be clearly seen but many of the structures comprising the organ of Corti are also visible. As can be seen in the motion pictures, the feet of the outer pillar cells are quite visible as is the tunnel of Corti. In the basal turn, as shown in Fig. 3 the feet of the inner pillar rest on the bone of the spiral osseous lamina and so are not visible The cuticular rods of Deiters cells and the striations on the basilar membrane are also visible, although they do not always readily show up in the motion picture because the field of focus is on the blood vessels.

Despite the fact that the view of this vessel is through fluid and by a combination of reflected and transmitted light, the structures and blood flow are very clearly revealed. The interest, so far has been in the normal blood flow

Fig 3 (A) Photomicrograph of the conventional histological view of the organ of Corti in the extreme basal region behind the round window where the basilar membrane is available for view through the microscope. The spiral capillary (*SC*) is quite prominent. The feet of the inner pillar cell rest on the tympanic lip of the spiral osseous lamina and the feet of the outer pillar cell re directly above the spiral capillary (B) The view of the basilar membrane and spiral capillary (*SC*) as seen through the microscope and photographed by the motion picture camera. *Tun* tunnel of Corti *ESOL*, edge of spiral osseous lamina *RWM* round window membrane *OPC* outer pillar cell *PVS* perivascular space *RBC* red blood cell floating free in perilymph, *BL*, bony ledge.

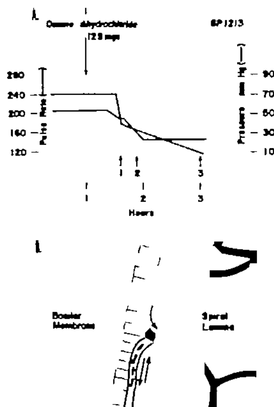


Fig. 4 (A) Blood pressure and pulse rate in 500 g animal given quinine dihydrochloride in the dosage of 250 mg/kg. The arrows at 1 2, and 3 indicate times at which motion pictures were taken. The flow in one branch of the "T" junction gradually stops. Although this animal went on to die others showed return to normal capillary flow after the animal metabolized the drug. Capillary flow rate is unrelated to pulse rate or carotid blood pressure (B) Enlarged tracing of the "T" junction in the above animal portraying the behavior of capillary blood flow in one branch of the "T" junction as seen in the motion picture.

and the effects of a substance, quinine dihydrochloride, known to produce a temporary hearing loss and which has been shown in surface preparations to cause obstruction to the flow in these small capillaries.

RESULTS

The objective of this investigation was to develop the technique for observing, in the living state the spiral vessel within the layers of the basilar membrane, and to provide a means for recording its reaction under various conditions. Eventually the goal is to assess the functional capacity of the organ of Corti in relation to these changes. In view of this, these results describe two things: (1) the view of the normal spiral vessel, and (2) its reaction to quinine dihydrochloride

These results are accompanied by a motion picture film in which the flow of blood through these vessels is seen with framing speeds as high as 128 frames per second.

Fig. 3 A is a conventional histological view of the organ of Corti in the very basal turn behind the round window membrane. It is to be noted that in this region, the basilar membrane is quite narrow and the Hensen cells do not stand up in a mound as they do in the upper turns. The position of the pillar cells shifts from that in the upper turns, so that the feet rest on the bone of the spiral osseous lamina. The spiral vessel lies beneath the foot of the outer pillar cell, and the tunnel lies above the region of the basilar membrane between the spiral osseous lamina and the spiral vessel. In the basal turn there is a bony ledge which extends *along the underside of the spiral ligament*. This and the spiral osseous lamina outline the basilar membrane, and under proper lighting conditions, the structures of the organ of Corti can be seen.

As described in the procedure, the round window membrane is removed so that the basilar membrane is exposed directly Fig. 3 B shows the view as seen through the monocular microscope and clearly reveals the spiral capillary bordering the tunnel the other side of which is the spiral osseous lamina. This particular capillary happens to be supplied by two branches coming out from the spiral osseous lamina. The outer pillar cell foot and perivascular space around the capillary are also visible. The labelled red blood cells are free floating cells in the perilymph. Ordinarily these are not present, but in this particular animal, there was some blood flowing from the cut edge of the bone that had been removed to expose the basilar membrane

As nearly as can be determined, the rate of flow through these capillaries, in an animal in good physiological condition and having suffered very little blood loss during the course of the surgery is about 320 μ per second. This is very rapid and it would appear that what this blood supply lacks in volume it makes up in velocity

The diameter of this vessel is fairly constant, around 10 μ The erythrocyte of the guinea pig averages about 7.5 μ and so only one red blood cell gets through this vessel at a time Because there is hardly room in this vessel for the red blood cells and plasma at the same time, there are gaps of plasma flowing between groups of red cells.

In monitoring the flow of blood through these vessels, it was necessary first of all to run control animals, in which the preparation was maintained in the experimental condition, over long periods of time to see if the flow continued at the normal rate The observations in these control animals have been reported earlier (Lawrence 1970) When the animal was artificially respirated properly with little blood loss during the operation, the blood flow was maintained constantly over a period in excess of 8 hours.

The motion picture films of this spiral capillary demonstrate quite clearly the cessation of blood flow following the injection of the quinine dihydrochloride (250 mg/kg) One branch of a "T" junction seems to become occluded and the blood flow ceases. This is not related to the changes that may occur in blood pressure or pulse rate, and as time goes on, the capillary blood flow becomes normal again as the toxin is metabolized.

Fig. 4 A is a graph reconstructed from the raw graphic data recording the pulse rate and blood pressure from an animal who was injected with the quinine dihydrochloride This animal was a 500 g animal given 125 mg of the drug. At the end of 1 hour after the beginning of recording, with the animal under stable condition of a blood pressure of 70 mm of mercury and a pulse rate of a little above 200 the

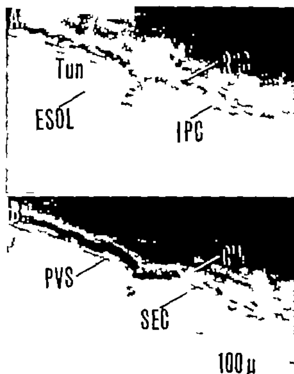


Fig 5 Views of the spiral vessel taken with the still picture camera showing typical stoppage of the flow in one branch of a "T" junction. In (A), a few red blood cells can be seen getting through the occlusion and in (B) the jamming of red cells at the site of the occlusion can be seen. *Tun.*, tunnel *RBC* red blood cell *ESOL*, edge of spiral osseous lamina *IPC* inner pillar cell *PVS* perivascular space; *SEC* swollen endothelial cell. This could also be a pericyte; *CW* capillary wall.

drug was administered. The arrows on the graph numbered 1 2, and 3 are times when motion pictures were taken of the capillary flow At the end of 1 hour and 40 min, the blood pressure underwent a fairly rapid drop to 30 mm of mercury and continued a slow progression downwards. The pulse rate slowed to about 140 and then remained constant. This animal went on to die, so in subsequent animals, the dosage was spread out over a period of time In this latter instance, the blood flow recovered. However in the animal reported here the blood flow ceased at position 2, and the manner of flow is indicated in Fig. 4 B

Occlusion seems to arise at or near a "T" junction. As the capillary comes out from the

spiral osseous lamina, and turns to flow along the basilar membrane in both directions, it seems to develop a swollen endothelial cell in one direction or the other As indicated by the arrows in Fig. 4 B there is some pumping of the blood in both directions, which is more closely related to respiratory movement than pulse rate. As is shown in the motion picture the swollen cell obstructs the vessel and occasionally a cell is able to squeeze by but for the most part, the flow ceases.

Fig. 5 shows a typical picture of the cessation of the flow due to the blockage in a spiral capillary In Fig. 5 A, the edge of the spiral osseous lamina and tunnel are visible, as are the feet of the inner pillar cells. The capillary comes out from the spiral osseous lamina to a junction, and then goes in both directions along the basilar membrane. However in one direction, the cells are seen singly and the individual red cells spread out with plasma in between. In Fig. 5 B the swollen endothelial cell can be seen along with the cell wall and now a clear plasma space following packed red cells is visible. It is possible that this cell is a pericyte. Future clearer photography should reveal the true nature of these cells.

As this technique is perfected, it is anticipated that the relationship between occlusion of these vessels and the drug will be more clearly portrayed. Motion pictures taken at 128 frames per second are still not fast enough to slow down the normal flow but with adequate surgical exposure and use of the synchronized Chadwick-Helmuth strobe light, it may be possible to go to somewhat higher speeds.

Future experiments are designed to measure oxygen distribution in the organ of Corti, the electrical potentials, and the effects of various other kinds of drugs.

ACKNOWLEDGMENTS

I acknowledge the very valuabl technical assistance of Miss Maxine Clapper the precise art work f M Gerry Evans, and the talented supervision of the photography by M Max Tedford, director of photography at the Kresge Hearing Research Institute, University of Michigan.

RESUME

Les vaisseaux en spirale de la membrane basilaire semblent être extrêmement importants pour le bon fonctionnement de l'organe de Corti, mais il été toujours difficile d'y accéder pour les étudier Cette communication décrit une manière d'aborder l'étude de ces vaisseaux sur l'animal vivant. Des films du flot sanguin sont pris sur un animal normal, et la cessation du flot capillaire sous l'influence de divers médicaments ototoxiques est demontrée.

ZUSAMMENFASSUNG

Die Spiralgefässe der Basilarmembran scheinen für die Funktion des Cortischen Organes höchst wichtig zu sein, aber wegen ihrer Unzugänglichkeit liessen sie sich bisher schwerlich untersuchen. Die vorliegende Arbeit beschreibt eine Methode, wie diese Gefässe in dem lebenden Tier studiert werden können. Filme über die Durchblutung bei dem normalen Tier werden gemacht und das Aufhören des Blutstromes unter dem Einfluss verschiedener ototoxischer Drogen demonstriert.

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DISCUSSION

H S Spoendlin. Mr Lawrence accords great impor tance to the vas spirale for the oxygen supply f the organ f Corti However in the adult cat the vas spirale is normally obliterated in the lower basal turn and I wonder whether it is the entire vascular arcade system of the osseous spiral lamina rather than the one as spirale under the basilar membrane for the supply of the organ of Corti?

Cath Smith. Perlman & Kimura have studied circulation on the lateral wall f the cochlear duct and found flow time in the arterio-venous arcades to be faster than that in the stria vascularis. I wonder if Mr Lawrence would comment on the relative rate of flow in the vas spirale. Is it comparable to that of arterio-venous arcades or that of stria?

E. Borghesan. Le vase spiral chez le foetus est très important tandis que chez l'animal adulte est très petit. Peut-est que ce fait est lié avec la circulation périlymphotic laquelle chez le foetus est très active tandis que chez l'animal adulte se reduit?

M Lawrence (Reply) to Mr *Spoendlin.* In the guinea pig and in man, as Mr Axelsson has described, the capillaries of the basilar membrane and of the tympanic lip persist throughout the basal turns. But in the very basal region the basilar membrane becomes quite narrow with the tunnel of Corti just adjacent to the lip of the spiral osseous lamina. Thus it is not essential that a capillary remain out on the basilar membrane. Oxygen can be supplied to the organ of Corti by the capillaries on the tympanic lip of the spiral osseous lamina. Another group of capillaries that may serve an important function for the organ of Corti but of which we know nothing are those of the limbus.

To Miss *Smith* The rate of flow in the capillaries of the basilar membrane is comparable to the most rapid flow observed in the arterio-venous arcades of the spiral ligament by Perlman & Kimura. The flow is considerably more rapid than that observed in the stria vascularis.

To Mr *Borghesan.* The spiral vessel does appear to be much larger in the foetus than in the adult and this may well be related to the metabolic requirement of the developing organ of Corti. It has been shown by Hilding that in some breeds f mice that become deaf 2 to 4 weeks after birth degeneration f the organ f Corti follows disappearence of the spiral capillary The organ of Corti develops norma ly as long as the spiral capillaries are present.

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ELECTRON MICROSCOPY IN HEAD AND NECK ONCOLOGY

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Abstract A systematic investigation of tumours of the ear nose and throat has been carried out, with particular attention to tumours arisi g from the squamous epithelium salivary glands, glomus jugulare, neurogenic tumours, muscle tumours granular cell myoblastoma, malignant lymphoma. Some of their main ultrastructural features were described and summarized. Surface cellular changes and changes in keratinization and alteration of the basal membrane were noted in squamous cell carcinoma of the larynx and oesophagus. Amorphous granules characterize the granular cells of granular cell myoblastoma. An incidental finding f this tumour in the trachea, associated with carcinoma of the larynx, is described. Striated myoblasts or filamentous myoblasts were found to be of assistance in the differential diagnosis of anaplastic sarcoma. Virus particles were found in case of malignant lymphoma of the tonsil The age of miracles in electron microscopy has come—and gone. W do not expect that electron microscopy or similar spectacular new method, might solve, and solve easily without any effort, the mystery of malignant change of the cell. W ha reached a period of rational hopes, based upon intelligent cooperation and the systematic study of surgical material, with electron microscopy stimulating our curiosity and assisting in the more accurate classification and or diagnosis, as potent ally f light microscopy

Progress in histopathology upon which much *surgical diagnosis is based has been outpaced* by the advances in immunology biochemistry and microbiology

The methods used in histopathology have their origin in the last century when the discovery of the achromatic compound microscope revolutionized morbid anatomy and indeed biology in general. We seem to have relied too long on the denaturation of protein by formal saline, that we call fixation on paraffin embedding; and even the staining methods are not unlike those used by Ehrlich, employing the dyes synthetized, you might be interested to know by his uncle Weigert (Gardner 1970)

An eminent English pathologist, Dr Gardner at a recent symposium on Automation and data processing in pathology" has called for a revolution in histopathological technique to rescue it from the doldrums.

Thus great Collegium counts among its Fellows many a revolutionary who has employed some of these techniques with considerable success, e.g. in the advancement of our knowledge of the inner ear

Whether we agree with Gardner or not, as pathologists we can best contribute by the collecting of basic data, since even a computer can provide the correct answers only on the basis of adequate data processing. We have carried out, in cooperation with our surgical colleagues at the Royal National Throat, Nose and Ear Hospital in London and under the auspices of the Cancer Campaign for Research, a systematic study of the ultrastructural features of neoplasms and other diseases affecting the head and neck.

It is worth remembering that two types of epithelial lesions may exist, with exactly the same morphological appearance, and one is benign, while the other inevitably progresses to frank invasive cancer It has been suggested that, to distinguish them it might be helpful to define cancer in terms of what tumour cells do rather than of what they look like (Huxley 1958) This would include the study of the chemical and metabolic activity of cells in normal and abnormal epithelium. I submit that electron microscopy may help to reveal some

of the subtle changes in cells undergoing neoplastic change.

Table II is a summary albeit incomplete of our principal observations. It is followed by a dual clinical and microscopical summary of 3 patients whose neoplasms we have examined, illustrating the assistance derived from electron microscopy

MATERIAL AND METHODS

TABLE I *Material (1964–1969)*

Total number of neoplasms including	546
Squamous cell carcinoma	?01
Adenocarcinoma	3
Salivary gland tumour	1
Adenolymphoma	4
Malignant melanoma	3
Neurilemoma	11
Granular cell myoblastoma	3
Rhabdomyosarcoma	7
Glomus jugulare tumour	

CASE REPORTS

Association of ca. larynx and granular cell myoblastoma of the trachea

Mrs A. S. (under the care of Prof. Harrison) 50 years of age when admitted complaining of hoarseness since August, 1969 She has smoked 20 cigarettes a day

Direct laryngoscopy revealed a large ulcerat mass arising from the left ventricular band, involving the left vocal cord, the left aryepiglottic fold and the base of the epiglottis.

Following super voltage irradiation, the lesion became much smaller

A total laryngectomy was performed on 14th May 1970

At operation (Mr A C. Gilchrist) in addition to a large neoplasm of the epiglottis, a small granular mass was found on the posterior wall of the trachea, considered to be, also on frozen sections, a secondary carcinoma.

Histology of the laryngeal growth showed well differentiated squamous cell carcinoma. The tracheal mass was identified under the electron microscope as a granular cell myoblastoma. Fig. 2 shows the characteristic inclusions in two granular cells.

Recurrent rhabdomyosarcoma

Mr A. S (under the care of Prof. Harrison) 43 years of age, presented in 1963 with an enlarged mass behind his left ear for 1 month—not painful, except on head movement.

Excision biopsy of soft mass 1 × 1/2 lying within the insertion of left sternomastoid The diagnosis malignant lymphangioma or fibrosarcoma was subsequently revised to embryonal rhabdomyosarcoma.

Radiotherapy 6880 given to left neck over 6 weeks. The tumour recurred several times and light and electron-microscope studies have been carried out, the last one in July 1970

Histopathology Light microscope diagnosis caused some difficulty and electron microscopy was of real diagnostic help to me in this case.

As in experimental rhabdomyosarcoma (Friedmann & Bird, 1969) several types of myoblasts have been identified. Fig. 3 shows a well-differentiated (Type IV) myoblast with fully developed striated myofibrils.

TABLE II *Summary of certain relevant findings*

Squamous cell carcinoma
Basement membrane
- Vesiculation and signs of breakdown (Fig. 1).

Cell
- Cell membrane: loss of desmosomes, thickening, phocytosis
- Nucleus: typism
- Organelles: degeneration
- Lysosomes: enhanced activity
- Other inclusions

Other neoplasms
Inert inclusions
- Crystalline: salivary gland tumour plasmocytoma
- Para-crystalline malignant melanoma
- Granular glycogen-ubiquitous; neurosecretory-paraganglioma
- Amorphous: granular cell myoblastoma (to be published in detail elsewhere)
- Filamentous: long-spaced collagen in schwannoma, also Menière's disease
- Myofilamentous differentiated rhabdomyosarcoma; undifferentiated rhabdomyosarcoma

Live inclusions
- Virus: malignant lymphoma



Fig 1 Oesophageal biopsy. Squamous cell carcinoma. Note vesiculation and disruption of the basement membrane. 28 000.

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MATERIAL AND METHODS

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Rhabdomyosarcoma	7
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- Myofilamentous: differentiated rhabdomyosarcoma, undifferentiated rhabdomyosarcoma

Live inclusions
- Virus: malignant lymphoma



Fig. 5. Recurrent rhabdomyosarcoma of the neck. Well-differentiated myoblast (Type IV). Note striated myofibrils and glycogen granules. 70 000



Fig 4 Malignant lymphoma tonsil. Inclusion body near nucleus filled with virus particles. 70 000.

Viruses in a malignant lymphoma

The virus aetiology of some neoplasms is not in doubt, but conclusive evidence in human cases is sparse

An antigen prepared from cultured cells from a case of Burkitt's lymphoma was used by Old et al. (1966) in serological studies. This tumour was selected because it contained large numbers of virus particles. It is clear that virus particles should be looked for in neoplasms of the head and neck, e.g. in nasopharyngeal carcinoma, malignant lymphoma.

Vacuolation of nuclei, nucleoli and cytoplasm occurs in malignant cells, but seldom contain virus particles. The following case might, therefore, be of some interest (Friedmann, 1969).

The patient was a woman of 43 years, born in Guyana. She was referred to this hospital with the pathological diagnosis of lymphoma of the tonsil, and was examined by Mr K. Rotter on August 21st, 1969 At this time there were hard matted glands on the left side of the neck, and the left tonsil was greatly enlarged, with a central necrotic area. After her admission to the hospital, the cervical nodes and both tonsils continued to increase in size and several enlarged lymph-nodes appeared on the right side of the neck. Biopsy confirmed the diagnosis of malignant lymphoma-reticulosarcoma.

A small piece of tissue was fixed in 3% buffered (pH 7.2) glutaraldehyde and embedded in araldite. On electron-microscopy the cytoplasm of the neoplastic cells was shown to contain multiple intracellular vacuoles or inclusion-bodies these contained large numbers of viruses, 60–70 mμ in diameter (Fig. 4) The virus particles have a double membrane and are surrounded by a corona of smaller units.

RÉSUMÉ

Une investigation systématique des tumeurs de l'oreille, du nez et du gorge été faite. On a fait attention spéciale tumeurs trouvant leur origine dans l'épithélium squameux le lymphome malin les tumeurs de la glande salivaire les tumeurs du glomus jugulare les tumeurs neurogéniques; le myome des cellules granulaires; rhabdomyosarcome. Leurs caractéristiques ultracellulaires seront discutées ec référence particulière à quelques-unes des caractéristiques suivantes: Des changements de la surface cellulaire et des changements de kératinisation et la modification de la membrane basale ont été observés dans le carcinome des cellules squameuses du laryn et de l'oesophage. On a pas trouvé des irus dans les papillomes juveniles. Des particules ressemblant les irus ont été notées dans le lymphome malin de l'amygdale laryngienne. La présence et la signification du collagène qui forme les soi-disant Corps de Luse dans le système nerveux et dans les neurilémmomes de la branche vestibulaire du huitième nerf ont été étudiées. Des lymphomes malins sont été étudiés avec référence particulière à l'occurrence des particules ressemblant les irus. On peut conclure que la microscopie électronique est un complement de grand valeur de la microscopie ordinaire dans l'identification des néoplasmes de la tête et du cou.

ZUSAMMENFASSUNG

Diese Mitteilung beruht auf den Ergebnissen einer systematischen Untersuchung der submikroskopischen Zellstruktur der Tumoren des Halses, der Nase und der Ohren. Besonderer Wert wird auf die Befunde in Tumoren des Retikulo-endothelialen Systems, Rhabdomyosarkom Krebs des Kehlkopfes, Gekornt zelliges Myoblastom gelegt. Die elektronenmikroskopische Untersuchungsmethode hat teilweise bisherige lichtmikroskopische Befunde bestätigt oder erweitert, anderseits, hat sie zu einer genaueren Diagnostik gewisser Tumoren beigetragen.

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DISCUSSION

J Wersall Was Mr Friedmann able to differentiate between myofibrils and fonofibrils in tumours of, for example, the parotid gland?

F A Soov Mr Friedmann mentions in his résumé that he has not been able to identify virus-like particles in juvenile laryngeal papillomas. We have this year in our laboratory identified virus-like particles in two patients under the work of Dr William Boyle. These particles were found only in the very young, small and new papillomas, not in the older or larger papillomas. The particles measure 500 to 900 Å and resemble Type A virus particles.

S Podvinec The Japanese colleagues, with whom I have the honour to collaborate found recently that malignant cells in cancerous tissue show no evidence of cell-to-cell communication as opposed to normal cells in electron-microscopic studies. What is Mr Friedmann's opinion on this matter?

I Friedmann (Reply) to Mr *Wersall*. You have put your finger on an important difficulty We have distinguished in experimental rhabdomyosarcoma four types of cells. The undifferentiated type of myoblast is most difficult to identify

To Mr *Soov* You recalled a patient who had been treated first as a boy of 5 under the late Prof. Ormerod. Now about 20 years later and after about 50 removals of multiple recurrent papilloma and in about 20 specimens examined under the EM no viruses (or virus-like particles) have been identified. Maturation of the cell might be the reason of the absence of viruses.

To M *Podvinec* I could refer only to the mechanism of contact inhibition

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NASOPULMONARY MECHANICS–EXPERIMENTAL EVIDENCE OF THE INFLUENCE OF THE UPPER AIRWAY UPON THE LOWER

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Abstract Nasal obstruction from whatever source, anatomic or infectious, causes measurably increased lower airway resistance. This has been verified by (1) establishment of control groups of normal subjects for each of our successive studies, (2) oral and nasal measurements of pulmonary function in subjects with nasal and laryngeal obstruction, with postoperative observation, in some cases, for evaluation of changes in respiratory mechanics following corrective surgery (3) establishment of baseline measurements of normal and abnormal pulmonary function in the human, (4) partitioning of total pulmonary resistance in the human and dog, and (5) long term observation of various types of surgically created nasal obstruction, as well as induced inflammation of the nasal cavities and paranasal sinuses in dogs, in order to study the effect of these conditions upon the function or mechanics of the lower airway

Observations of the pulmonary mechanics of the upper and lower airway systems are sparse We have felt that the etiology of certain adverse changes in pulmonary function in subjects with upper airway obstruction can hardly be explained by local pathology alone but must be studied through an integrated approach to the entire respiratory system. We have measured these various parameters of respiration during both nasal and oral breathing, and have documented measurable changes in the mechanics of respiration in subjects with marked upper airway pathology Additional evidence has been gathered through controlled experimental investigations in the dog.

In these studies we have attempted to relate obstructive upper airway pathology including chronic rhino-sinusitis, but without lower air way disease, to changes in pulmonary mechanics in the human subject.

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REVIEW OF LITERATURE

Precise objective determination of the effects of upper airway obstruction upon the dynamics of respiration has been a fairly recent observation. The work of earlier investigators, such as Paulson (1882) Lüscher (1930) and Sercer (1930) who were among the first to suggest the respiratory influence of the nose upon the mechanics of respiration, and Rohrer (1915) whose k and k_2 constants for laminar and turbulent flow still remain valid, has been well documented in our earlier publications (Ogura et al., 1964 1965 Togawa & Ogura, 1966) Although many fine reports have been made by rhino-physiologists dealing with the lower airway significant data are lacking in regard to the relationship of upper airway obstruction to changes in lower airway function. Proctor (1964) and Williams (1968) studied the physiologic function of the upper airway in addition to air conductance. Masing (1967) reported an investigation into the upper air way airstream. Gray (1967) discussed the deviated nasal septum and its influence on the physiology and diseases of the nose and ears, while Brown (1967) measured resistance of the nasal passages to the movement of air in relation to increased mechanical and reflex resistance Drettner (1970) has dis-

Table I. *Anatomical and physiological classification of nasal obstruction*

Classification	Description of the nose
Normal nose	
0	Straight septum, perfectly good airw y
1	Straight septum, slight enlargement of turbinates, no nasal obstruction
2	Deviated septum, with or without minimal nasal obstruction[a]
Abnormal nose	
3	Moderately deviated septum, septal spur unilateral narrowing of middle meatus, fixed unilateral nasal obstruction
4	Anterior deviation of nasal septum Unilateral impaction by the septum, and unilateral upper lateral cartilage collapse, with open airway on the side. Moderate obstruction[b]
5	Same as classification 4, but with bilateral upper lateral cartilage collapse and moderate bilateral nasal obstruction
6	Severe anatomic bilateral septal deviation of the nose with severe bilateral nasal obstruction from septal and upper lateral cartilage impingement[c]

Minimal nasal obstruction; means mild symptomatic unilateral or bilateral, and intermittent.
[b] Moderate nasal obstruction, means obstruction is frequent or complet on one side and incomplete and good airway on the other side
Severe nasal obstruction means nearly constant and bilateral.

ssed the pathophysiological relationship be ween the upper and lower airways.

Tomari & Widdicombe (1968) have reported on reflexes elicited by mechanical stimulation of the respiratory tract and the co-existance of upper and lower airway inflammation has been discussed by Kartagener & Stuki in 1962, and by Logan et al. in 1965 Some cases of cor pulmonale in children due to upper respiratory tract infection have been reported (Menashe et al., 1965 Luke et al., 1966 Levy et al., 1967) None of these reports, however deal with the exact parameters of our investigations on pul monary function.

METHODS

We have proposed basically that a definite neurophysiologic relationship exists between the upper and lower airway systems, and that obstructive pathology of the upper airway whether by anatomic structure or by chronic disease, may alter the mechanics of respiration. In order to establish the validity of this premise we have studied various parameters of respiratory me chanics in humans and animals.

Our procedure consisted of (1) establishing control groups of normal subjects for each of our successive studies, (2) masking oral and nasal measurements of pulmonary function in subjects with nasal and laryngeal obstruction, with postoperative observation, in some cases, for evaluation of changes in respiratory mechan ics following corrective surgery (3) establishing baseline measurement of normal and abnormal pulmonary function in humans, (4) partitioning of total pulmonary resistance in humans and dogs, and (5) long term observation of various modalities of surgically created nasal obstruction, as well as experimentally produced inflammation of the nasal cavities and paranasal sinuses in dogs, in order to study the effect of these conditions upon the function or mechanics of the lower airway

Our investigations have been concerned with mechanics of breathing measurements, with evaluation made during both nasal and oral breathing. These studies have been performed according to the methods of pulmonary physiologists (Mead & Whittenberger 1953), and consist of ventilatory studies (VC, timed VC and MVV) pulmonary compliance and resistance measurements using the esophageal balloon method (Mead et al., 1955) functional residual capacity measurements, and the separation of pulmonary resistance into its components, air way and tissue resistance, using a volume type body plethysmograph (Dubois et al., 1956) We have standardized our procedures and results through control groups, with repetition of studies on the same individuals in order to analyze day to day variations, and evaluation of anthropomorphic differences, as well as sex and age differences. Early in our investigations we established an Anatomical and Physiological Chart for classifying various degrees of nasal

obstruction (Table I). We have continued to use this chart in all our studies.

Partitioning of total pulmonary resistance in the human is performed in the accepted manner. Pressure in the subglottic space is obtained through a square end blunt needle (Gauge 17) equipped with a sharp stylus, inserted through the cricothyroid cartilage under local anesthesia. Pleural surface pressure is measured indirectly using an esophageal balloon.

RESULTS AND DISCUSSION

Nasal obstruction

Our original test observations were made on 170 clinical subjects of both sexes, with and without nasal obstruction. These early investigations consisted of ventilatory studies and pulmonary compliance and resistance measurements, using an esophageal balloon, with measurements made during both nasal and oral breathing. The information obtained from these studies was inconsistent due to early difficulties in adaptation and technique however results were encouraging in that we were able to observe some definite trend toward changes in the mechanics of breathing in subjects with nasal obstruction as compared with normal subjects.

When experimental error was considered to be very minimal we made a series of advanced studies so as to numerically increase our earlier observations for statistical accuracy. In all our investigations we have noted a decrease in compliance and an increase in pulmonary resistance, during oral as well as nasal breathing, in the majority of all subjects tested with relatively severe nasal pathology. These values revert to the normal range in approximately the same number of cases, following successful surgical correction of the obstruction.

In order to clarify the etiology of these observed changes it became necessary to separate pulmonary resistance into its components, airway and tissue resistance. A volume type body plethysmograph was used for these investigations on 12 healthy medical and dental students. Analysis of the data confirmed our earlier findings, and showed also that airway resistance was significantly increased during oral breathing in subjects with obstructive nasal pathology (Unno et al., 1968)

Pre- and postoperative measurements of nasal obstruction

This study was extended to 54 subjects for further numerical and statistical significance (Ogura et al. 1968) with particular emphasis placed on pre- and postoperative measurements and results. Case histories were presented showing favorable reversal of the mechanics of breathing in cases with nasal obstruction within four to five months following corrective nasal surgery. Total pulmonary resistance, even when measured orally appears to increase with increasing degrees of nasal obstruction. The fact that these values revert to normal range, in most instances, following successful nasal surgery would indicate that reversible changes occur. Since measurements during mouth breathing are entirely separate from the nose we suggest that anatomic or physiologic changes must occur in the lower airway secondary to pathologic changes in the upper airway

Partitioning of total resistance in the human

We have partitioned total pulmonary resistance in four persons with normal noses and two others with abnormal noses (Shimada & Ogura, 1970). Four of the cases represent what we believe to be normal values of resistance for three airway components, during nasal breathing, i.e., the nose, larynx and lower airway and two components during oral breathing, consisting of the upper airway (mainly the laryngeal region) and the lower airway. We found nasal and upper airway resistance quite variable during mouth breathing and careful evaluation is necessary. The contribution of the upper and lower airways to total pulmonary resistance was analyzed in the 6 cases (Tables II, III). During nasal breathing the percent contribution of each region in the normal cases is (1) nose 53.7% (2) laryngeal region, 17.4% and

Table II *Resistance of each component during nasal breathing*

	Nose		Laryngeal region		Lower airway	
	Insp.	Exp.	Insp.	Exp.	Insp.	Exp.
Normal						
B. W	1 43	1.01	0.42	0.33	0.58	1.38
L. K.	0.81	0.73	0.31	0.38	0.57	1 73
T E.	1.06	0.93	0.31	0.41	0.60	0.98
R. S.	1_5	1.31	0 42	0.25	0.70	0.97
Mean	1 14	1.00	0.37	0.34	0 61	1.27
S. D	0.26	0.24	0.06	0.07	0.06	0.36
Per cent	53 7	38.34	17 4	13 02	28.77	48.65
Abnormal						
G S.	2.90 (52.7 %)	2.84 (47.9 %)	1.37 (24 9 %)	0.91 (15.3 %)	1_3 (22.3)	2.18 (36.8 %)
G J	4 76 (70.7)	6 67 (63 7)	1.05 (15 6 %)	1.64 (15 6 %)	0.92 (13 7)	2.17 (20.7)

(3) lower airway 28 77 % during inspiration. During expiration the contribution is (1) nose, 38.34 % (2) laryngeal region 13 02 % and (3) lower airway 48 65 %

During mouth breathing the contribution of the upper airway is 55 % during inspiration and 34 10 % during expiration. Lower airway contribution is 44 01 % during inspiration, and 65 68 % during expiration.

Values for nasal and upper airway resistance are lower than those reported by other investors. We attribute this to the careful selec and evaluation of our test subjects from

Table III *Resistance of each component during mouth breathing*

	Upper airway		Lower airway	
	Insp	Exp	Insp	Exp.
Normal				
B. W	1 08	0.84	0.57	0.84
L. W	0.54	0.43	0 60	1.35
T E.	0.82	0.69	0 46	0.88
R. S.	0.42	0.34	0.70	1.38
Mean	0.72	0.58	0.58	1 11
S.D	0.30	0.23	0 10	0.29
Per cent	55	34.10	44 01	65.68
Abnormal				
G S.	1.34 (41.5)	1 11 (33 7 %)	1.89 (58.5)	2.18 (66 3)
G J	0.79 (29 9 %)	0.89 (28.5 %)	1.85 (70.1)	2.23 (71.5 %)

the standpoint of the degree of anatomic nasal obstruction. The small population of this study is being enlarged. Greater numbers are needed before definite conclusions can be made.

Pre and postoperative studies of laryngeal obstruction in man

Since any investigations into the effects of upper airway obstruction must include laryngeal pathology we have measured changes in respiratory mechanics in a number of cases with laryngeal obstruction These subjects always show extremely high preoperative measurements of pulmonary and airway resistance dur ing both oral and nasal breathing, although nasal anatomy may be quite normal. Post operative studies are being undertaken at this time to observe the effects, over a long term of radiation therapy as opposed to definitive procedure. These results are being analyzed and will be reported later

Baseline measurements for normal and abnormal pulmonary function

For some time we have believed that functional baseline measurements for pulmonary function could be established and used practically for evaluating normal and abnormal pulmonary dynamics since our studies indicate that objective determination of the work of breathing

Table IV *Changes in resistance after surgery*

Δ is symbol of pre and postoperative difference

					Mouth			Nose		
No.	Subject	Sex	Age	Degree of obstruct. Pre—Post	ΔR_p	ΔR_A (cm H_2O/LPS)	ΔR_T	ΔR_p	ΔR_A (cm H_2O/LPS)	ΔR_T
1	D L.	♂	28	3 → 1	−0.30	−0.40	0.10	−1.00	−0.98	−0.62
2	V K.	♀	43	4 → 1	−3.10	−1.12	−1.98	−2.00	−0.36	−[illegible].24
3	W J	♂	31	4 → 2	−0.30	−0.28	−0.02	−1.20	−0.24	−0.96
4	R. S.	♂	32	4 → 2	−0.20	−0.43	+0.23	0.30	0.48	−0.18
5	O R.	♂	16	4 → 1	−0.20	−0.74	0.54	−1.30	−0.86	−0.44
6	J M	♀	20	4 → 2	0.50	+0.23	0.27	−0.50	0	−0.50
7	P K.	♀	29	4 → 1	−0.10	−0.48	0.38	−0.80	−0.69	−0.11
8	V G	♀	33	4 → 2	0	−0.37	0.37	−1.00	−0.79	−0.21
9	J S.	♀	25	4 → 2	−0.20	−0.17	−0.03	−0.30	+0.71	−1.01
10	L. C.	♂	14	4 → 1	−0.55	−0.05	−0.50	−1.20	−0.36	−0.84
11	D W	♂	17	5 → 2	−0.80	−0.43	−0.37	−6.20	−5.16	−1.04
12	N. Z.	♂	48	5 → 2	−0.70	−0.59	−0.11	−4.50	−0.69	−4.21
13	J K.	♂	43	5 → 2	−0.20	−0.85	+0.65	−3.13	−4.72	+0.59
14	C. S.	♀	23	5 → 2	−1.30	−0.44	−0.86	−3.50	−1.44	−2.06
15	S. G	♀	31	5 → 4	+0.10	−0.19	0.29	−6.20	−1.94	−4.26

should be determined through mechanics of breathing measurements, rather than from rhinometric evaluation alone. In order to establish these baseline measurements we have examined a total of 97 clinical subjects (Ogura et al., 1968). A normal control group was established, and 15 subjects were tested both pre and postoperatively (Table IV).

As a result of these studies we were able to classify three distinct groups: (1) normal, (2) borderline, and (3) nasal obstruction, and to establish baseline measurements for function in each of these categories (Figs. 1, 2). These have been used routinely in our clinical laboratory for evaluating normal and abnormal pulmonary function. These measurements have

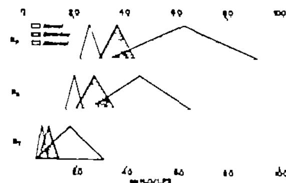


Fig. 1. Pulmonary resistance in relation to nasal obstruction (nose breathing).

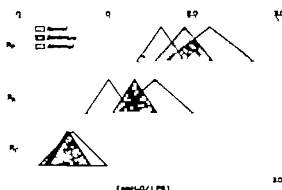


Fig. 2. Pulmonary resistance in relation to nasal obstruction (mouth breathing).

been especially useful in the "borderline" cases of nasal obstruction. These subjects may or may not be candidates for nasal surgery and should be carefully evaluated from the standpoint of significant changes in pulmonary mechanics in order to determine criteria for surgery.

We are also investigating the approximately 15% of the patients who appear to get no symptomatic improvement following surgery although the anatomic obstruction is materially reduced. These persons continue to complain of shortness of breath, mouth breathing and associated symptoms. We have postulated several possible reasons: (1) minor organic





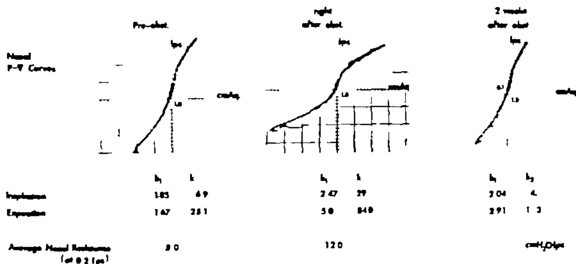


Fig 3 Unilateral obstruction and nasal resistance (dogs).

changes in the lung, which were not detectable re or postoperatively at the time from physi- and roentgenologic examination, (2) persis-]nasal obstruction from other causes (non rgic vasomotor obstruction) and (3) normal readings of airway resistance, during oral breathing, *preoperatively*

At this point in our investigations data strongly suggest that measurements of airway resistance during mouth breathing present important criteria for evaluation of normal and abnormal pulmonary function. When airway resistance measurements are normal during mouth breathing, even though nasal resistance is high, surgery for function is usually contraindicated (Ogura et al., 1968) Improvement in the mechanics of breathing cannot occur in these cases, since it is already normal, and symptomatic complaints persist in spite of a substantial reduction in nasal anatomic obstruction. We are testing the validity of these observations on greater numbers for significance.

Our *objective findings to date strongly indicate* that upper airway pathology may be an important contributor to early chronic changes in the mechanics of respiration, and therefore *significantly affect the normal function of the lower airway*

Confirming evidence of our clinical observations is being gathered through studies on dogs, under normal and experimental situations. These investigations are designed to relate specifically to similar conditions encountered in the human subject, and to emphasize the clinical application of our investigations.

Distribution of airway resistance along the respiratory system in dogs

Using dogs, we have partitioned airway resistance into its three major components, the nose, larynx and lower airway in order to observe

Fig. 4. Bilateral obstruction and nasal resistance (dogs).

the actual distribution of airway resistance along the entire respiratory tract, and at the same time determine the normal contribution of each segment to total pulmonary resistance (Ohnishi & Ogura, 1969). The resistance of the upper airway both nose and larynx, changes constantly during breathing, due to the changes in instantaneous flow rate.

We have established a method for accurate measurement of nasal resistance in the dog, with minimal artifact from the muscular activities of the soft palate and pharyngeal muscles. Simultaneous measurements of the nose, larynx and lower airway permit calculation of instantaneous values of resistance at each respiratory segment. Rohrer's constants were calculated for each segment, and the pressure flow relations were expressed in Rohrer's formula. As expected, the nasal passages accounted for the greatest source of flow resistance (79.2%) of total resistance. Resistance of the larynx was 5.9% and that of the lower airway 14.9% at mid-flow normal respiratory flow rate in the dog (0.25 LPS). No significant differences were noted in k_1 and k_2 values in the nose during the inspiratory and expiratory phases.

In the larynx, however there were significant differences in the values between the inspiratory and expiratory phase. The ratio was considerably greater during expiration, due to the adduction of the vocal cords in this phase. We expect to apply this knowledge to the human subject, to determine the distribution of flow resistance in the normal respiratory tract, and the disturbance of normal distribution in the subject with severe upper airway pathology.

Effects of chronic nasal obstruction upon the function of the lungs in the dog

We have suggested that long standing upper airway pathology may cause disturbance

Fig 5 Changes in resistive work (dogs).

in normal respiratory performance However general changes which may occur in the lower airway are not yet clearly understood.

We have proposed to investigate whether chronic upper airway obstruction causes any change in the functional and mechanical aspects of the lungs, and if this is shown to be true what is the nature of the change.

In order to study this we created, surgically various degrees of nasal obstruction in full grown mongrel dogs, two with unilateral obstruction, three with partial bilateral obstruction, and one, with complete obstruction on side and partial obstruction on the other observed changes in the mechanical properties of the lungs in these dogs over a period of four months (Ohnishi et al., 1970) Analysis of pressure flow relations in the dogs with unilateral obstruction showed that though nasal resistance was elevated immediately following the creation of the nasal obstruction, it was compensated for in a relatively short period of time (Fig. 3) These results appear to correlate well with our borderline" type of nasal obstruction in the human subject. Although some difficulty in nasal breathing may be present, both human and dog appear to compensate readily to this degree of obstruction, and little or no adverse effects are noted upon the mechanics of respiration.

However changes in the mechanical properties of the lung were observed in most of the cases with high nasal resistance i.e. bilateral obstruction (Fig. 4) It appears that disturbance of the normal physiological function of the upper airway does lead to changes in normal lung function. Longer observation will be needed.

Effects of chronic inflammation upon the mechanics of the lung in dogs

Chronic rhinitis and sinusitis have been produced in a group of dogs with unilateral nasal obstruction, who showed no obvious changes in the mechanics of breathing during the period of observation. This study is in progress (Ohnishi & Ogura, 1970) and will be reported later Some observations can be made at this time

In contrast to our findings in the dogs with simple nasal obstruction, where nasal resistance varied according to the type of created obstruction, as well as to the extent of compensation on the part of the nasal structures, the dogs with nasal and paranasal inflammation tended to show greater uniform nasal resistance. The type of nasal obstruction created in these dogs seems to have little significance in relation to values in nasal resistance.

This might be attributable to the near total failure of the turbinates to compensate for elevated nasal resistance in the presence of severe pathologic changes in the mucous membrane and underlying tissues. General changes indicate to positive increase in resistive work during the period of observation (Fig. 5) how-

Fig 6 Changes in elastic work (dogs).

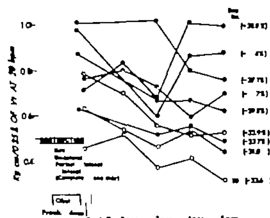


Fig 7 Changes in total work (dogs).

ever the elastic work during the same period was greater (Fig. 6), thus total lung work tended to decrease with nasal inflammation (Fig. 7). This may be interpreted as compensation for the increased work load of the respiratory muscles in order to overcome greater nasal resistance.

We suggest that changes in the variables, following surgical correction of the existing pathology is not a simple function of the degree of mechanical obstruction removed, since these changes are seen during oral as well as nasal breathing. The various parameters may be changed by a number of factors. Mechanical and neural (nasopulmonary) reflex mechanisms must be taken into account as probable causative effects.

We are planning advanced studies of the nasopulmonary and pulmonasal reflex pathways in our animal laboratory The greater pressure gradients which are necessary to move certain amounts of air into the lungs to exceed increased nasal resistance may primarily work as a stimulus to afferent endings, mechano receptors and baroreceptors.

In the past we have gathered evidence from various sources and methods, all directed toward demonstrating the influence of upper air way obstruction upon increases in lower air way resistance. The basic mechanisms in these phenomena remain largely unexplored We have suggested a relationship between severe upper airway pathology and the possible early development of chronic obstructive lung disease. This unified concept must be investigated through continued clinical studies in greater number and confirming neurophysiological studies in dogs, in order to explain dynamic changes in pressure-flow and compliance elastance of the airways, when severe upper airway pathology is evident.

RESUMÉ

Nous discuterons les aspects de nos études du nez obstrué dans l'altération de la résistance du canal d'aérage inférieur 1 Evaluation des examens utilisés pour distinguer entre les malades qui sont aidés par une opération et ceux pour qui une opération est sans valeur 2. Influence quantitative du nez sur l'action des poumons. 3 Division de la résistance respiratoire chez les humains et les chiens. 4 Contribution d'obstruction ou d'infection des voies aériennes supérieures à la résistance des voies aériennes inférieures.

ZUSAMMENFASSUNG

Der derzeitige Stand unserer fortlaufenden Untersuchungen über den Einfluss von Obstruktionen der Nase auf den Widerstand der unteren Luftwege wird geschildert. Im einzelnen werden folgende Punkte besprochen. 1 Statistische Validation von Tests zur Beurteilung, ob durch chirurgische Eingriffe eine Besserung fur den Patienten erzielt werden kann. 2. Normale Bezugswerte über den Einfluss der Nase auf die Mechanik der tieferen Luftwege. 3 Unterteilung des Gesamtwiderstandes der Luftwege beim Menschen und beim Hund. 4 Tierexperimentelle Ergebnisse über den Einfluss von Obstruktionen und Infektionen der oberen Luftwege auf den Widerstand der unteren Luftwege.

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DISCUSSION

I Padovan. It would be interesting to know

(*a*) which equipement was used? (*b*) with how many channels? ( ) how the obtained curves are adapted for computorization? (*d*) how an international standardization of the rhinomanometric curve could be achieved?

J Tonndorf Mr Ogura mentioned cases in which surgery although producing an apparently wide nose, did not alleviate the patient s complaint. The reason for that lies in the fact that the nasal airflow is at the border between laminary flow and turbulent *DkP* In nose that is too wide there is eddying and often turbulence. Whereas in laminary airflow Ohm's law prevails, in turbulent flow resistance varies with the square of the airflow a fact that soon leads to respiratory difficulties.

J H Ogura (Reply) to Mr *Padovan.* The electrical equipment is obtained from Sanborn Co. in Massachusetts. Standardization of rhinometric measurements has not been established but represents charge *of the committee of the American Academy of Otol.*

To Mr *Tonndorf* I am quite aware of your thesis and that turbulent flow accounts for increased airway resistance however I did make measurements through the nose and separately through the mouth.

HORMONALE UND ELECTRONENOPTISCHE NASENRACHEN FIBROM STUDIEN

Rosemarie Albrecht, K. Schubert und K. Küttner

Aus der Hals-Nasen-Ohrenklinik der Friedrich-Schiller-Universität und der Deutschen Akademie der Wissenschaften zu Berlin Institut für Mikrobiologie und experimentelle Therapie Jena Abteilung für Steroidforschung Jena DDR

Abstrakt. Die Bestimmung der spontanen Ausscheidung von Hormonen und Hormonmetaboliten im Harn zeigt bei Nasenrachenfibromträgern keine typischen Besonderheiten. Selbst bei kräftiger Stimulierung mit Testosteron, Gonadotropin und Follikelstimulierendem Hormon weicht die endocrine Reaktion im Vergleich zu Gesunden gleichen Alters nur wenig und nicht sicher spezifisch ab. Trotzdem ist die therapeutische Wirkung von Sexualhormonen unbestreitbar wobei Oestrogene nach eigenen Beobachtungen den Androgenen überlegen sind. Ihre Wirkung ist jedoch insgesamt begrenzt. Dagegen erwies sich das Di-methyl-di-aethyl-Stilboestrol allen Präparaten überlegen. Es kann lange Zeit appliziert werden und hat keine stärkeren Nebenwirkungen. Die Geschwulstrückbildung ist in Einzelfällen bedeutend, so daß es bei sehr ausgedehnten Tumoren eine echte Hilfe ist. Electronenmikroskopisch finden sich charakteristische Strukturen, unter denen bisher unbekannte Kerneinschlußkörperchen besonders auffallen. Vielleicht sind sie für das Nasenrachenfibrom spezifisch, aber noch ist nicht bekannt, was sie bedeuten.

Trotz einer Reihe von Hormonstudien und trotz mancher Mitteilungen über Therapieerfolge mit Hormonen beim juvenilen Nasenrachenfibrom erschien uns die hormonelle Auslösung oder Steuerung dieser Geschwulst, möglicherweise durch Gonadenhypo- oder dysfunktion, keineswegs erwiesen und eine massiv dosierte Hormontherapie im typischen Fibromalter ohne Kenntnis der ursächlichen Störungsstruktur durchaus anfechtbar.

Aus diesem Grunde haben wir im vergangenen Jahrzehnt gerne die Möglichkeit der Zusammenarbeit mit der Abteilung für Steroidforschung des Instituts für klinische Mikrobiologie und experimentelle Therapie der Deutschen Akademie der Wissenschaften genutzt und uns als Ziel gesetzt, mit allen sinnvoll erscheinenden Mitteln der Klinik und des Laboratoriums zu prüfen, ob bei Fibromträgern eine spezifische hormonelle Abweichung nachweisbar sei.

Teilergebnisse wurden von uns verschiedentlich mitgeteilt (Albrecht & Schubert, 1965 Schubert & Albrecht, 1963 Schubert et al., 1965) Wenn wir aber erst heute zu einer abschliessenden Berichterstattung kommen, so ist es Ausdruck der Schwierigkeiten, die Aufgabe befriedigend zu bewältigen. Die Ursachen liegen in der Seltenheit des Nasenrachenfibroms, zumindest in Europa und in der Tatsache, Hormonstudien gerade in einem Altersabschnitt durchzuführen, der hormonell eine Phase stürmischer Entwicklung darstellt und ungewöhnlich reich an zeitweiligen individuellen Abweichungen ist, so daß Normwerte kaum existieren.

Der jetzige Bericht hat die Aufgabe:

1 abschliessend über Reifestatus und endocrine Leistung der von uns untersuchten Fibromträger zu informieren,
2. unsere Schlußfolgerungen zur Hormontherapie darzulegen und
3 spezielle electronenmikroskopische Befunde zur Diskussion zu stellen.

Zu 1 Systematische Bestimmungen des Reifegrades und der spontanen Steroidausscheı-

dung im Harn sowie nach hormoneller Belastung wurden bei 12 Nasenrachenfibromkranken verschiedenen Alters sowie einem Verdachtsfall, bei dem sich der Tumor später als ungewöhnlicher Choanalpolyp erwies, durchgeführt. Steroidvergleichsanalysen wurden zusätzlich an 26 gesunden Kontrollpersonen verschiedener Altersgruppen der Pubertät vorgenommen. Klinisch wurde neben den üblichen Aufgaben der Diagnostik und Therapie der Reifegrad der Patienten durch Kontrolle des Skelettsystems, der sekundären Geschlechtsmerkmale und bei älteren Patienten des Spermiogramms bestimmt. Das Knochenalter war in 5 Fällen altersentsprechend, sonst fand sich eine Akzeleration durchschnittlich von 3 J (Prof. E. Haessler Univ. Kinderklinik, Jena.) Das äußere Genitale zeigte in keinem Fall Besonderheiten. Die sekundären Geschlechtsmerkmale waren unauffällig mit Ausnahme eines Patienten, der angedeutet das Bild eines pubertalen Eunnuchoidismus bot (Univ. Hautklinik Jena, Prof. Langhoff). Bei allen älteren Patienten waren die Spermiogramme regelrecht. Nur bei einem 20 jähr. Patienten fand sich eine Asthenohypospermie (Univ. Hautklinik Jena, Prof. Langhoff). Bei diesem wurde auch als [illegible]ocrinologische Rarität erstmalig eine große [illegible]ge Progesteron im Harn nachgewiesen.

Die Steroidausscheidung wurde vor und nach Testbelastungen mit Testosteron, Choriongonadotropin (HCG) und follikelstimulierendem Hormon (FSH) durchgeführt. Auf die Testbelastung legten wir größten Wert in der Vorstellung, daß leichtere Störungsbilder deutlicher zu Tage treten müssen, wenn das Endocrinium zu ungewöhnlichen Leistungen gezwungen wird.

Einzelheiten über Belastungsmodus sowie Untersuchungstechniken wurden von Schubert (1957, 1963) in früheren Arbeiten dargelegt, so daß hier nur die Ergebnisse gebracht werden.

1. *Corticosteroide*

Die Ausgangswerte der Gesamtcorticosteroide lagen bei Fibromträgern vielleicht etwas niedriger als bei Gesunden. Bei Belastung mit 1 000 E HCG zeigte sich ein Anstieg der Corticosteroide, der bei Fibromträgern durch höhere Dosierung von 6 000 E nicht weiter anstieg im Gegensatz zu den Gesunden.

2. *Gesamt 17-Ketosteroide*

Sie stiegen unter Testosteron bzw. Gonadotropin erwartungsgemäß an, wobei die Hauptmetabolite Androsteron und Ätiocholanolon am stärksten ansprachen. Bei Erhöhung der HCG-Dosis auf 6 000 E stiegen die Werte bei Gesunden weiter an im Gegensatz zu den Fibrompatienten.

3. *Oestrogene*

Es wurden keine besonderen Ausscheidungswerte im Vergleich zur gesunden Kontrollgruppe gefunden mit Ausnahme eines Patienten, der durch FSH HCG besonders hohe Oestrogenwerte erreichte.

4. *Gonadotropin*

Die Gonadotropinausscheidung war nur bei einem Teil der Fibromträger erhöht.

5. *αβ-ungesättigte Ketosteroide*

Die Testosteronausscheidung zeigte bei Fibromträgern Altersunterschiede. Bei 12–13 Jährigen waren die Werte altersbedingt niedrig. Oberhalb 16 J. lagen die Werte mit ca. 30 μg /24 Std. vielleicht etwas niedrig. Die Androstendionausscheidung zeigte keine altersmäßigen Beziehungen und blieb bei 50 mg Testosteronbelastung im Vergleich zu Gesunden auffallend niedrig.

Die vorgelegten Beobachtungen und Ergebnisse haben trotz großen Aufwandes und hoher Kosten keine umstürzenden Erkenntnisse gebracht. Wir glauben jedoch einige Details als gesichert ansehen zu dürfen.

Es zeigt der Nasenrachenfibrom-Patient keine Störung des Knochenalters. Die Ossifikation ist entweder altersentsprechend oder auch beschleunigt, niemals aber retardiert. Die äuße

ren Geschlechtsmerkmale zeigen keine eindeutige Verzögerung. Das Spermiogramm ist in der Regel normal. Gelegentliche Auffälligkeiten, wie in unserem Krankengut, erlauben keinen spezifischen Bezug auf das Nasenrachenfibrom.

Unter den Hormonbefunden erscheint durch die Arbeiten besonders italienischer Kollegen zusammen mit unserem Untersuchungen gesichert, daß beim Fibromträger keine Störung der gonadotropen Hypophysenfunktion besteht. Die Ausscheidungswerte im Urin wurden entweder erhöht (Carbonara & Salonna, 1958 De Judicibus, 1961 Maurizi, 1963) oder in normaler Menge vorgefunden (eigene Untersuchungen), niemals dagegen vermindert. Es steht dieses in guter Übereinstimmung mit der Skelettreifung.

Über die Steroidausscheidung beim Fibromkranken gehen im Schrifttum die Angaben stark auseinander und leider wird die Tatsache, daß wir mit 17 Ketosteroiden oder $\alpha\beta$ Hydroxyketosteroiden u. s. f. keine individuellen Substanzen, sondern Stoffgruppen unterschiedlicher Herkunft bestimmen, oft außer Acht gelassen. Es ist nicht statthaft, allein aus Werten der 17 Ketosteroidausscheidung im Harn auf eine Gonadeninsuffizienz zu schließen, insbesondere nicht in einem hormonell so labilen Alter der Nasenrachenfibromträger

Dennoch besteht kein Zweifel daran, daß erhöhte Steroidausscheidungswerte bei Fibrompatienten niemals gefunden wurden. Die Diskussion geht um normale oder erniedrigte Werte (De Judicibus, 1961 Migliorini, 1964 Patterson, 1965 Karatay et al., 1963 1969)

Unsere *allgemeinen Steroidresultate liegen* innerhalb der physiologischen Schwankungsbreite der jeweiligen Jahrgänge vielleicht mehr in Richtung der unteren Grenzwerte Aber vielleicht ist es interessant, daß die Corticosteroide und die Gesamt 17 Ketosteroide bei Gonadotropinbelastung ab 6 000 E nicht weiter anstiegen. *Der markanteste Befund unserer vorliegenden* Untersuchungsreihe liegt in der Gruppe der $\alpha\beta$-ungesättigten Ketosteroide bei den Androstendionwerten, die bei 50 mg Testosteronbehandlung im Vergleich zu Gesunden ohne altersmäßige Beziehung auffallend niedrig blieben.



Abb 1 Großes Nasenrachenfibrom mit ausgedehntem endocraniellem Einbruch durch die vordere Schädelbasis.

Die Abb. zeigt den Zustand nach intensiver Stilboestrolbehandlung, auf welche der Tumor nur mit mäßiger Rückbildung reagiert hat.

Insgesamt müssen wir aber klar aussprechen, daß beim juvenilen Angiofibrom keine spezifischen Veränderungen im Steroidhaushalt vorliegen, die sich mit den gegenwärtigen Methoden der Endocrinologie aus der Variantenfülle *der Pubertät signifikant abgrenzen ließen.* Eine Theorie der Unter oder Dysfunktion der Gonaden, läßt sich nicht aufrechterhalten. Nach unseren Untersuchungen dürfte man maximal wenn man absolut Schlußfolgerungen ziehen will — von einem geringfügig verzögerten Ansprechen der Gonaden bei Fibrompatienten *sprechen, das möglicherweise nur allgemeiner* Ausdruck einer allgemein belastenden Erkrankung ist, zu denen das Nasenrachenfibrom letztlich wohl zu rechnen ist.


Abb 2 Gleicher Fall. Geschwulstrückbildung unter Zytonal.

Zu Irgendwelche Hinweise für eine kausale Hormontherapie des Nasenrachenfibroms geben uns Hormonanalysen nicht. Wenn wir dennoch aus klinischer Empirie wissen, daß sowohl Testosteron als auch Oestrogen eine reduzierende Wirkung auf den Tumor haben können, so muß diese Wirkung meines Erachtens als unspezifische Mesenchymreifung gedeutet werden.

Im Schrifttum halten sich die Anhänger der Testosteron- und der Oestrogenbehandlung etwa die Waage. Einige sehr interessante Beobachtungen besagen, daß der Tumor unter Androgenen aber auch im Wachstum angeregt werden kann (Boedts, 1940 Johnson et al. 1966). Von Schiff (1959) wissen wir daß er Oestrogen für wirksamer hält.

Eigene Teste haben die Möglichkeit, das Nasenrachenfibrom hormonell zu beeinflussen, bestätigt, wobei Oestrogene weit überlegen waren. Es sind die Hormone jedoch nur eine gewisse Zeit und bis zu einer bestimmten Dosierung wirksam. Für das Stilboestrol möchten wir die beste Wirkung mit einer Gesamtdosis von ca. 3 000 mg in 4 Monaten angeben. Über diese Zeit und diese Dosis hinaus haben wir keinen sicheren Nutzen mehr gesehen

Für Fibrome mit exzessiver Wachstumstendenz und besonderen Komplikationen, die eine radikale operative Entfernung auch bei heutigen operativen Möglichkeiten schwierig oder vital bedrohlich erscheinen lassen, möchten wir auf ein besonderes Präparat aufmerksam machen und seinen Versuch empfehlen. Es handelt sich um das Tetranatriumsalz des 4 4-Dihydroxy-$\alpha\beta$-diaethylstilbendiphosphats, das im Organismus als Diäthylstilboestrol wirksam sein soll mit hoher zytostatischer Wirksamkeit.

Es wird bei metastasierendem Prostatacarcinom vielfach verwendet und ist in der DDR als „Zytonal im Handel, in der BRD unter dem Namen „Hovan

Es ist hinsichtlich seiner Nebenwirkungen auf die Gonaden und Mammae nicht sonderlich aktiv wird über lange Zeit vertragen und beeinträchtigt trotz sehr hoher Gesamtdosis die


Abb 3 Gleicher Fall. Zustand nach operativer Exstirpation des Resttumors.
Deutliche Reossifikation der Schädelbasis.

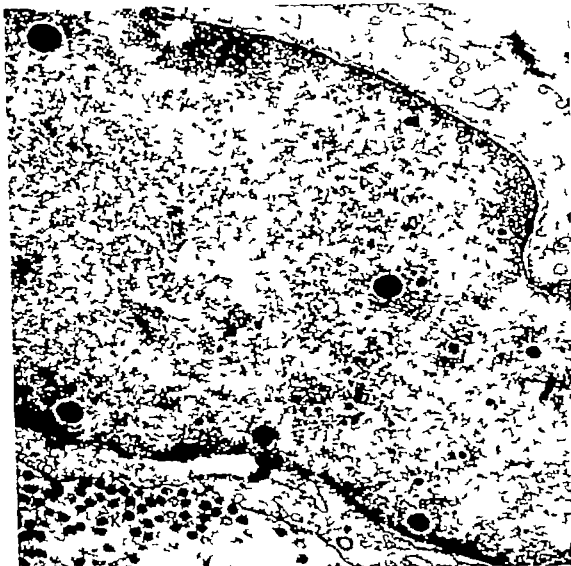


Abb. 4 Unterschiedlich große elektronendichte Einschlußkörper (E) mit umgebendem schmalem hellem Saum vorwiegend kernmembrannah in einem Fibrozytenkern (N). Angrenzend kollagene Fasern (K). Originalvergrößerung 12000 fach/Nachvergrößerung auf 36500 fach.

postoperative Heilungsfähigkeit nicht. Es gelang uns in einem Falle mit ausgedehnten intracraniellem Einbruch nach Versagen von Stilboestrol, Testosteron, Co[60] Bestrahlung, den Tumor alleine mit diesem Präparat soweit zur Rückbildung zu bringen, daß die operative Entfernung des Restes einwandfrei möglich wurde. Der maximal mögliche Effekt war allerdings erst nach einer Gesamtdosis von 155 g erreicht.

Zum Wirkungsmechanismus dieses Präparates beim Angiofibrom können wir noch nichts sagen, da wir noch keine Gelegenheit hatten zu prüfen, wie sich die sauren Phosphatasen in dieser Geschwulst verhalten.

Zu 3. Neben den klinischen und hormonellen Untersuchungen wurde in jedem Falle auch das Tumorgewebe selbst unter verschiedenen Fragestellungen untersucht.

Der Übergang zu electronenmikroskopi-

schen Untersuchungen erfolgte eigentlich mit dem Ziel, eine etwaige Virusgenese zu prüfen. Sie bestätigten und vertieften die bisher licht mikroskopisch erhobenen Befunde, brachten aber auch völlig neue zytomorphologische Aspekte. Diese konzentrierten sich vor allem auf die sogenannten Stromazellen des Nasenrachen fibroms, auf die Fibrozyten und Fibroblasten sowie deren oft schwer abgrenzbare Vorstufe. In den Zellkernen dieser Elemente finden sich multiple unterschiedlich große electronen dichte Einschlußkörper die von einem schmalen hellen Hof umgeben werden. Diese Kerneinschlüsse erscheinen in den Fibroblasten über dem gesamten Kernbereich verteilt und von besonders auffallend wechselnder Größe während sie sich in den Fibrozyten zunehmend in der Nähe der Kernmembran anordnen. Sie sind nicht zu verwechseln mit Kerneinschlußkörper die durch 2 bis 3 konzentrische Doppelmembranen mit multiplen electronendichten Granula im Zentrum gekennzeichnet sind, wie wir sie in unseren Materialien auch fanden. Diese 2. Form wird in den verschiedensten Zellformationen sowohl unter normalen, als auch unter pathologischen Verhältnissen gesehen und sind für Nasenrachenfibrome sicher nicht spezifisch (Bouteille et al., 1967 Henry & Petts, 1969)

Unabhängig voneinander wurde die erste Form der Kerneinschlußkörper nach der uns zugänglichen Literatur 1966 erstmalig von Svoboda & Kirchner erwähnt, und 1969 von Seifert anlässlich eines Kongresses in Miskolz, Ungarn, vorgetragen und von McGavran et al. (1969) als ultrastrukturelles Charakteristikum der Stromazellen des juvenilen Nasenrachen fibroms, die nicht im Stroma des normalen Nasenrachens oder in Nasenschleimhautpolypen vorkommen, publiziert. Einschliesslich unseres Materials (4 Fälle) (Albrecht et al., 1970) sind in der Literatur bisher 15 entsprechende electronenmikroskopische Untersuchungen bekannt, die alle die „intranuclear dense bodies" aufwiesen. Wir können zusätzlich mitteilen, daß sie keine Unterschiede zeigen gleichgültig ob der Patient rein chirurgisch oder mit Tele cobalt oder intensiv mit Hormonen vorbehandelt wurde

Die Bedeutung der Einschlußkörper in den Stromazellen ist z. Z. noch unklar Viralen Ursprungs sind sie sicherlich nicht. Weitere Studien werden zeigen müssen, ob sie wirklich für das Nasenrachenfibrom spezifisch sind oder ob sie auch bei anderen Geschwülsten auftreten und welche Deutung sie eines Tages erhalten werden.

SUMMARY

I nasopharyngeal fibroma the determination of the spontaneous excretion of hormone and hormone-metabolites in the urine reveals no typical peculiarities. Even on fficaceous stimulation with testosteron, gonadotropin and folliclestimulating hormone, there is minimal and not surely specific difference in the endocrinal response compared with normal persons of equal age. Nevertheless we cannot deny the thera peutic ffect of sex hormones. In our observations estrogene proved to be more effective than androgens though its effect is limited too. The best of all seems to be: di-methyl-di-aethyl-stilboestrol. We can apply it for long time without unwanted side-reactions. The tumor-involution can be considerable, so that in cases of wide spread fibroma it can be of valuable help. In the electronmicroscope we find some characteristic structures of which peculiar nuclear inclusive bodies are the most striking ones. May be they are specific findings in nasopharyngeal fibroma. But up to date we do not know anything bout their importance.

RESUMÉ

La détermination de l'excrétion spontane d'hormones et d'hormones métaboliques dans l'urine ne montre pas de particularités typiques chez des porteurs fibronasopharingiens. Même vis-à-vis d'une stimulation vigoureuse avec Testosteron, Gonadotropin et hormone stimulant des follicules, la réaction endocrine diffère seulement peu et n est pas exactement spécifiée comparée aux personnes saines du même âge. Malgré cela l'effet thérapeutique des hormones sexuelles est incontestable, quand même d' près des propres observations les œstrogènes sont supérieurs aux Androgènes Contre cela le Di-methyl-di-aethyl-Stilboestrol surpasse les autres préparations. On le peut ppliquer longtemps sans graves actions secondaires. La transformation régressive de la tumeur est considérable dans certains cas et par conséquence une véritable aide quand il s'agit des tumeurs très élargies. Moyennant du microscope électronique on peut constater des structures caractéristiques, parmi lesquelles se font remarquer avant tout des corps inclus substantiels, inconnus jusqu'à présent. Peut-être ils sont spécifiques pour le fibrome naso-pharyngien, mais on ne connaît pas encore leur signification.

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DISCUSSION

I Friedmann. Were there any female patients in your series?

R. Albrecht (Reply) to Mr *Friedmann.* Im eigenen Krankengut waren keine Frauen vertreten. In einem Fall von Mr Gauran erwies sich das Fibrom einer weiblichen Patientin d. NR s elektronenmikroskopisch.

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ESTERASES IN POST MORTEM INNER EAR FLUIDS

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Abstract. In electrophoretic analysis, using β-naphthyl acetate as substrate, post-mortem perilymph and endolymph, CSF and serum all showed 6 esterase fractions while NHS, diluted 1 10, had 4 fractions and CSF none. Fractions 1 and 2 were arylesterases while fraction 3 contained α_1-arylesterase acetylcholinesterase (AChE) and cholinesterase (ChE). Fraction 4 was ChE and fractions 5 and 6 were tissue esterases. ChE was the dominant fraction in serum while AChE dominated in inner ear fluids, brain tissue, acoustic nerve and acoustic tumour homogenates.

Carboxylic esterases represent an inhomogeneous group of enzymes which have relatively wide substrate specificities. In addition to cholinesterases which appear in the form of acetylcholinesterase (AChE) and non-specific cholinesterase (ChE) they include lipases and arylesterases as the main groups in human serum and tissues (Wilkinson, 1965) AChE is known to appear particularly in erythrocytes and nervous tissue, while ChE appears in plasma and in many tissues.

Gisselsson (1950) obtained evidence of acetylcholine splitting capacity of cod endolymph by recording slight acidification of a test mixture of endolymph and acetylcholine without physostigmin, the presence of which kept the pH unchanged at 7 0 Use of cod serum and cerebrospinal fluid (CSF) caused an even greater acidification. Stimulation of the physostigminized frog muscle by an acetylcholine perilymph or -endolymph mixture of cat ears caused a decrease in the muscle contraction as compared with pure acetylcholine solutions. Use of acetylcholine–serum mixture caused no contraction, testifying to a stronger esterase concentration in serum than in inner ear fluids. Perilymph of pigeons was found to have weaker effect than endolymph both of which possessed a much weaker acetylcholine splitting activity than serum. Similar relations as in pigeons were found with guinea pig perilymph and serum.

Gisselsson's experiments were an indirect demonstration of cholinesterases in the inner ear fluids and showed that these fluids contained acetylcholine splitting substances. However he could show no acetylcholine activity on leech muscle in direct methods using inner ear fluids of pigeon, rabbit, cat, guinea pig, and man. Prior stimulation by sound failed to bring any acetylcholine activity into the inner ear fluids. The results of these experiments were contrary to those of Martini (1941) who proposed that sound stimulation caused acetylcholine activity to appear in the pigeon perilymph.

Histochemically Schuknecht et al. (1959) showed that AChE normally present along the nerve fibres and in dense accumulations at the base of hair cells in the organ of Corti in the cat disappeared when the olivocochlear bundle was sectioned in the medulla. They concluded that the presence of AChE in the cochlea was dependent upon the integrity of this bundle. Electronmicroscopically Kaneko & Daly (1968) found that AChE activity uniformly appeared on the entire plasma membrane of the vesiculated nerve endings and of some preterminal axons. After streptomycin damage part of the AChE activity remained in the neighbourhood of destroyed hair cells (Schuknecht et al., 1959) while Marco & Lasal (1964) noted

Table I *Frequency of esterase bands in post-mortem inner ear fluids CSF and serum*

Percentages in parentheses

Test fluid	Esterase fraction 1	2	3	4	5	6
Perilymph, $N=31$	2 (6)	9 (30)	30 (97)	27 (87)	6 (20)	11 (36)
Utricular endolymph, $N=18$	2 (11)	4 (22)	17 (94)	7 (39)	3 (16)	6 (33)
Cochlear endolymph, $N=28$	2 (7)	5 (17)	27 (96)	17 (61)	8 (28)	9 (32)
Mixed inner ear fluids + cells, $N=17$	— —	5 (29)	15 (88)	11 (65)	4 (23)	1 (6)
CSF $N=14$	1 (7)	3 (21)	10 (71)	13 (92)	3 (21)	5 (35)
Serum, $N=8$	4 (50)	6 (75)	8 (100)	8 (100)	2 (25)	2 (25)
Normal human serum, $N=16$	6 (37)	12 (75)	2 (13)	16 (100)	— —	— —

disappearance of AChE from the cochlea. In the vestibular receptors Brunetti et al. (1964) and Rossi et al. (1964) presented evidence of acetylcholine accumulation by using cholinesterase inhibitors.

Our earlier studies using post-mortem human inner ear fluids included lactate and malate dehydrogenase analyses (Palva & Raunio, 1969 Palva et al., 1970) These enzymes in inner ear fluids had a greater resemblance to the respective patterns of CSF than to serum. The present studies with esterase activity were made to obtain further information of the pattern, and of origin of various components in inner ear fluids.

MATERIAL AND METHODS

The material used for individual studies consisted of inner ear fluids of 31 cadaver ears In addition 16 ears were used for pooled samples. Removal of the temporal bones was generally made during the first 24 hours and at the latest 48 hours post mortem. Serum was collected from great thoracic veins and CSF from the spinal canal. Collection of the inner ear fluids was done under the operating microscope using the methods described earlier (Palva & Raunio, 1967) As control solutions normal human serum (NHS) and normal CSF hemolysed erythrocytes, acoustic tumour tissue post-mortem brain tissue and acoustic nerve homogenates were used.

Electrophoresis was carried out in 1% agarose gel using barbiturate buffer at pH 8.6, following the procedure described by Wieme (1965) Field strength was 7–8 V/cm, duration of run 20 min, temperature of petroleum ether 12 C. The amount of test fluid used was 3 μl excepting endolymph, of which generally only 1–2 μl were available Serum was diluted to 1 10 whereas the other test fluids were used as such (Tables I, II).

Characterization of esterases was made by methods recommended by Uriel (1963) After electrophoresis the plates were kept for 60 min at room temperature in an incubation solution which was prepared as follows: 5 mg of β naphthyl acetate was dissolved in 0.5 ml of acetone to which 25 ml of 0 05 M Tris buffer at pH 7 4 was added. To this solution, 10 mg of diazo blue B was added and the solution filtered.

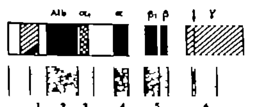


Fig 1 Schematic representation of the mobility f the 6 esterase fractions as compared th the normal protein pattern of serum.

Table II. *Dominating esterase fractions in post-mortem inner ear fluids, CSF and serum*
Percentages in parentheses

Test fluid	Esterase fraction 1	2	3	4	5	6
Perilymph, $N=31$	— —	— —	22 (71)	7 (23)	2 (6)	— —
Utricular endolymph, $N=18$	— —	— —	15 (83)	1 (6)	1 (6)	1 (6)
Cochlear endolymph, $N=28$	— —	— —	24 (86)	— —	4 (14)	— —
Mixed inner ear fluids and cells, $N=17$	— —	— —	13 (76)	— —	3 (19)	1 (6)
CSF $N=15$	— —	— —	8 (53)	7 (47)	— —	— —
Serum, N 8	— —	— —	2 (25)	6 (75)	— —	— —
Normal human serum, $N=16$	— —	— —	1 (5)	15 (95)	— —	— —

Various inhibitors were used for characterization of the enzymes. The plates were first placed for 15–30 min into the inhibitor solution and changed thereafter into the incubation solution which had the same concentration of inhibitor as the first solution. The inhibitors used were eserine, iso-OMPA, EDTA and BW284c51 in a concentration 2×10^{-6} and 2×10^{-4} 5 ml of 1% $CaCl_2$ solution added in 100 ml of stain solution was used as a co-factor for studying the fast components of esterase

Heat stability of the fractions was studied by incubating the plates in 55 C water bath for 20 to 60 min.

RESULTS

Table I gives the frequency of esterase bands in various post-mortem fluids and NHS In NHS four fractions were found ranging in mobility from prealbumin to α_2-globulin, while in normal CSF no activity could be demonstrated. In all post mortem fluids, six main fractions were obtained, the mobility of the two additional fractions being in the area of β and γ-globulins (Fig. 1) In post-mortem fluids, fractions 3 (α_1-globulin) was present in 71% of CSF in 97% of perilymph, and in all serum samples. This same fraction appeared in only 13% in NHS Fraction 4 was present in the inner ear fluids in varying percentages, least frequently in utricular endolymph (39%) then in cochlear endolymph (61%) and most frequently in perilymph (87%) NHS and post mortem serum showed this fraction in all cases, and it was present in 92% of post mortem CSF samples.

Fraction 1 the fastest band, appearing in the prealbumin region, was present in post-mortem inner ear fluids and CSF in about the same frequency (between 6 to 11%) while the serum and NHS figures were larger (50 and 37%) Similar distribution occurred for fraction 2, corresponding to the mobility of albumin, which was present in 17–30% in post-mortem inner ear fluids and CSF but in 75% in both serum groups.

Of the slower bands, fraction 5 (mobility of β-globulin) had equal frequency in all test fluids (16 to 28%) excepting NHS in which it was absent. The slowest band, fraction 6 (mobility of γ-globulin), appeared slightly more frequently than band 5 in the various test fluids (25 to 36%) excepting the mixture of inner ear fluids and cells, and NHS in which it was absent. Table II gives the dominating fraction in each test fluid. The pattern in various inner ear fluids is about the same, the dominating band being fraction 3 (71 to 86%) while fraction 4 was the next in frequency for perilymph (23%) (Figs. 2, 3) In CSF fraction 3 was slightly more frequent (53%) than fraction 4 (47%) but in NHS and post-mortem serum fraction 4 was dominant. Fraction 5 domi-

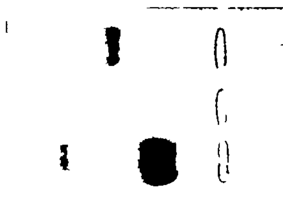


Fig. 2. Electrophoretic analysis of post-mortem perilymph, normal CSF and NHS. Fraction 3 dominates in perilymph and fraction 4 in NHS. CSF shows no esterases.

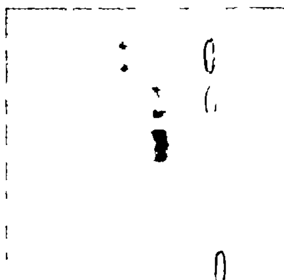


Fig. 4. Electrophoretic analysis of post-mortem endolymph, serum and NHS (*upper*), subjected to inhibition test with 2 10^{-4} M eserine for 30 min (*lower*). Fraction 4 is sensitive to eserine.

nated in 14% of cochlear endolymph and 19% of the mixture of inner ear fluids, while the latter and utricular endolymph showed fraction 6 dominating in one sample. None of the serum or CSF specimens had fractions 5 or 6 as dominant bands.

In the inhibition tests fraction 4 was sensitive to eserine (Fig. 4) and to iso-OMPA (Fig. 5) proving this band to be non-specific cholinesterase (Diegenbach, 1965). Slight inhibition of fractions 3 and 5 was also seen by both of these substances in post-mortem fluids. The use of BW284c51 showed partial inhibition of band 3 in post mortem fluids suggesting that this fraction consisted partly of acetylcholinesterase (Fig. 6). However in NHS neither BW284c51 nor eserine or iso-OMPA showed inhibition of fraction 3.

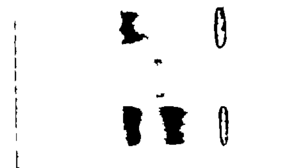


Fig. 5. Individual variation in endolymph analysis. Upper run shows endolymph of the right and the lowest from the left ear (NHS in the middle). Fraction 3 is strong in both endolymphs while in the left ear there is also strong fraction 4. This apparently represents contamination from serum.

All six fractions were inactivated by heating of the samples for 20 min in 55 C water bath. However in non-diluted post-mortem serum weak bands of fractions 3 or 5 remained suggesting the presence of small amounts of lipases. Addition of the calcium ions as a co-factor to NHS samples and inner ear fluids showed activity increase for fraction 1 and partial activation for fraction 3. Use of EDTA resulted in complete inhibition of fraction 1 but had no effect on fractions 2 and 3. Fraction 1 can thus be characterized as the prealbumin arylesterase. Fraction 2 had the mobility of albumin and being resistent to eserine and

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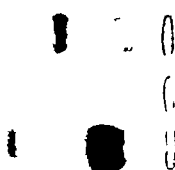


Fig 2 Electrophoretic analysis of post-mortem perilymph, normal CSF and NHS. Fraction 3 dominates in perilymph and fraction 4 in NHS. CSF shows no esterases.

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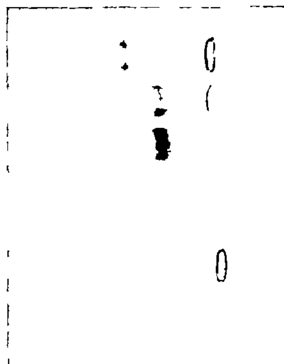


Fig 4 Electrophoretic analysis of post-mortem endolymph, serum and NHS (*pper*), subjected to inhibition test with 2 10^{-4} M eserine for 30 min (*lower*). Fraction 4 is sensitive to eserine.

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iso-OMPA is termed albumin arylesterase (Uriel, 1963 Wilkinson, 1965) while part of band 3 was designated as α_1 arylesterase

To obtain more information of various fractions, additional samples were analysed using α naphthyl acetate as substrate (Fig. 7) In some post-mortem fluids fraction 3 was divided into two bands. the whole fraction 3 was partially sensitive to eserine and iso-OMPA and the faster band also to BW284c51 This acetylcholinesterase containing band was strongest in several runs of pooled endolymph, somewhat less prominent in perilymph and post mortem serum samples when the amount of fluid was 2 μl and all samples undiluted. Further subdivision into 2 bands with this substrate was seen also for fraction 5 the slower of which was partially inhibited by iso-OMPA



Fig 5 Electrophoretic analysis f post-mortem sera diluted to 1 10 and to 1 25 and of a homogenate of nucleus caudatum (*uppe*), subjected to inhibition tests with iso-OMPA (2 10^{-4} M) for 30 min (*lower*). Fraction 4 is sensitive and fractions 3 and 5 are partially sensitive to the action of iso-OMPA.



Fig 6 Electrophoretic analysis of NHS, post-mortem serum and a homogenate of nucleus caudatum (*p-pe*), subjected to inhibition test with BW284c51 Only fraction 3 shows marked inhibition.

and eserine The fraction thus consisted of non specific cholinesterases and possibly of lipases.

Control experiments with brain homogenates of *nucleus caudatum, acoustic tumour* (Fig. 8) and acoustic nerve showed a homogeneous dominating fraction 3 migrating with the speed of the fast component in the divided fraction. This band was sensitive to BW284c51 In many specimens bands with the mobility of fractions 5 and 6 were also apparent. The same findings applied for hemolysed erythrocytes.

COMMENT

The results show that AChE activity appears associated with the electrophoretic fraction 3 a part of which may also consist of non-specific cholinesterases and α_1-arylesterases. In



Fig. 7 Electrophoretic analysis of NHS, post-mortem serum and CSF. The upper part has β-naphthylacetate and the lower α-naphthylacetate as substrate. Resolution of the fast end is poorer with the latter substrate.

NHS, as can be expected, fraction 3 consisted totally of the latter and there was no subdivision with α-naphthylacetate. In brain tissue, acoustic tumour and nerve, and in erythrocytes, the acetylcholinesterase fraction dominated. In all post-mortem inner ear fluids AChE activity formed the greater part of band 3 activity while in serum and CSF the acetylcholinesterase activity was a minor part of this fraction.

Fraction 4 ChE, was the dominating fraction in most NHS and post mortem serum samples and present in all specimens. In inner ear fluids, fraction 3 dominated while CSF assumed an intermediate position. Fractions 1 and 2, arylesterases, were found clearly more seldom in inner ear fluids and CSF than in either NHS or post-mortem serum. The presence of fractions 5 and 6 in all post-mortem samples but never in NHS can be regarded as indicating the liberation of tissue-esterases after death.

The esterase pattern in post-mortem fluids is quite complicated. Fraction 3 consists in brain and acoustic nerve tissue mainly of AChE which is also true for endolymph and perilymph. In inner ear fluids, this fraction includes some arylesterases and non-specific cholinesterases. The role of the latter two is more prominent in post-mortem serum samples.

It is evident that the esterase pattern of NHS with undoubted emphasis on ChE is retained in the post-mortem stage even if other fractions also become included. That this picture is not the same in inner ear fluids testifies further to the fact that these fluids derive their protein components from several sources and are no simple ultrafiltrates of plasma. The lack of esterases in normal CSF precludes this fluid as a possible source for esterase pattern in inner ear fluids. Analyses of brain tissue acoustic tumour and acoustic nerve homogenates are in conformity with the pattern seen in endolymph and perilymph. This may testify to the brain–inner ear pathways suggested in our earlier communication (Palva & Raunio 1957) although the dominance of AChE in inner ear fluids can also be explained by the specific



Fig. 8 Electrophoretic analysis of acoustic tumour extract, hemolyzed erythrocytes and NHS. Fraction 3 dominates in the upper two runs (fraction 5 represents hemoglobin in the second run) and fraction 4 in NHS.

function of the sensory cells in the nerve end ings of which the presence of AChE has been demonstrated with usual histochemical techniques. Whether AChE develops here or is car ried to the nerve endings with the axoplasmic movement cannot be answered at present.

ZUSAMMENFASSUNG

In post mortem Perilymphe, Endolymphe, Zerebrospinalflüssigkeit und Serum wurden 6 Esterasenfrak tionen mittels Elektrophoresis mit β-naphthylacetate als Substrat gefunden. In normalem Serum, 1 10 verdünnt, konnte man 4 Fraktionen demonstrieren und in normaler Zerebrospinalflüssigkeit waren keine Esterasen zu finden. Fraktionen 1 und 2 waren Arylesterasen, weil Fraktion 3 aus α_1-arylesterase, Acetyl cholinesterase (AChE) und Cholinesterase (ChE) be stand. Fraktion 4 war ChE und 5 und 6 Gewebeesterasen. ChE war die dominante Fraktion im Serum, weil AChE Fraktion in der Endolymphe und Perilymphe so wie in den Homogenaten us Gehirngewebe, Nervus acousticus und dessen Tumoren dominierte.

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ACETYLCHOLINESTERASE ACTIVITY IN THE VESTIBULAR SENSORY AREAS

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Abstract The localization of AChE activity was investigated in the cristae ampullares, utricle and saccule of chinchilla inner ear with the electron microscope by Karnovsky's method. The reaction was positive on the plasma membrane of the efferent nerve fibres and endings. The reaction product filled the synaptic gap between these endings and the afferent dendrites and nerve chalices. The reaction product was absent at the junction between the hair cell and the afferent nerve endings and chalices. The controls indicated that the reaction was due to AChE activity

Acetylcholinesterase (AChE) activity was histochemically demonstrated for the first time in the vestibular sensory areas by Dohlman et al. (1958) Later a more detailed localization was obtained with the aid of the electron microscope by Hilding & Wersäll (1962). The present research was undertaken to gain further knowledge on the localization of AChE activity in the vestibular labyrinth. For this, the labyrinth of chinchilla was studied at the electron microscope with the method suggested by Karnovsky (1964)

MATERIAL AND METHODS

Four adult chinchillas of both sexes and one adult guinea pig were anesthetized by endoperitoneal injection of sodium pentobarbital (35 mg/kg) The inner ear was prefixed in vivo by a 10 min perilymphatic perfusion of the fixative at room temperature. The animal was sacrificed and immediately after the temporal bones were quickly removed. The specimens were left for 3 hr at 0–4 C in the same fixative used for the perfusion. During fixation the cristae ampullares and the maculae utriculi and sacculi were identified and partly dissected.

Fixation was done in 3% glutaraldehyde or in 4% formaldehyde obtained from paraformaldehyde. The aldehydes were diluted in isotonic phosphate buffer prepared according to Millonig (1961) or in 0 08 M sodium cacodylate buffer After fixation the specimens were placed for 2 hr in the same previously used buffer and the dissection of the vestibular sensory areas was continued. Part of the specimens were then transferred into Karnovsky's (1964) incubation medium (substrate acetylthiocholine iodide, Hoffmann La Roche & Co Ltd., Basel) and incubated in toto for 40 min.

Other specimens were frozen and thawed and then incubated in the same way From other specimens 120 μ thick sections were obtained using a freezing microtome. The sections were put into Karnovsky incubation medium for 10 min.

After incubation the specimens were rinsed in a solution of 0 44 M sucrose and then post fixed in a 1–2% osmium tetroxide solution buffered according to Millonig (1961 with

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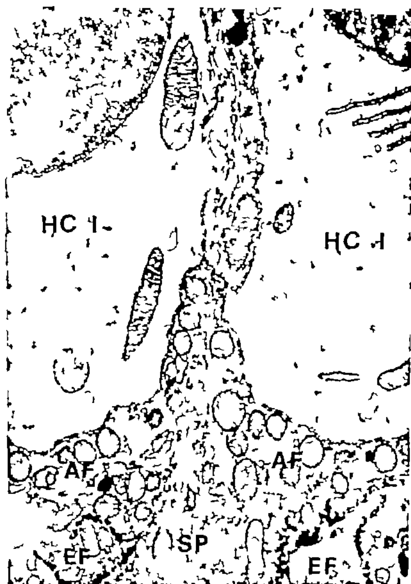


Fig 1 Crista ampullaris of chinchilla xamined with the electron microscope (formaldehyde-fixed material). The reaction product is localized on the plasma membrane of the fferent nerve endings (*EF*) in contact with the outer surface of the afferent nerve chalices (*AF*) *HC I* hair cell type I *SP* supporting cell 16 700.

s-collidine Dehydration was carried out in alcohol and embedding in Epon 812.

On part of the material the following controls were made (*a*) incubation in a medium lacking substrate (*b*) incubation with inhibitors ($1 \times 10^{-4} - 2 \times 10^{-4}$ M iso-OMPA, $1 \times 10^{-5} - 2 \times 10^{-}$ M BW 284 C 51) At these concentrations iso-OMPA selectively inhibits the unspecific ChE and BW 284 the AChE.

RESULTS

Similar results were obtained in the cristae ampullares and in the maculae utriculi and sacculi. To avoid repetition, a unique description is given.

The reaction product (Cu-ferrocyanide) was observed on the plasma membrane of the efferent nerve endings in contact with the afferent nerve chalices (Figs. 1 2 *b*) The reaction product filled the synaptic gap between these efferent synapses and the outer surface of the nerve chalices (Fig. 2 *b*)

The junction between the hair cells type I and the nerve chalices was always free of the reaction product (Figs. 1 2 *b*) At this level a finely granular intercellular material is interposed in a regular manner between the plasma



Fig. 2. (*a*) Crista ampullaris of chinchilla examined with the electron microscope (formaldehyde-fixed material). Axodendritic synapse below hair cell type I. The reaction product is present in the synaptic gap (*SY*). 60 000. (*b*) Utricle of chinchilla examined with the electron microscope (formaldehyde-fixed material). The reaction product fills the synaptic gap between an efferent nerve ending (*EF*) and the plasma membrane of the outer surface of the afferent nerve chalice (*AF*). The reaction product is absent in the gap between the hair cell type I (*HC I*) and the afferent nerve chalice (*AF*). 60 000.



Fig 3 Utricle of chinchilla examined with the electron microscope (formaldehyde fixed material). The product is localized on the non-myelinated nerve fibres (*NM*) below the basement membrane. *MY* myelinated nerve fibres, *SC* Schwann cell. × 4 000.

membrane of the hair cell and the nerve chalice (Smith, 1967 Hamilton, 1968) In order to be certain that the absence of AChE activity was not due to an inadequate penetration of the medium in this gap we made some trials on frozen and thawed material and on sections cut using the freezing microtome By these techniques, severe morphological alterations were found in the neuroepithelium. However this procedure did not produce a different localization of the enzymatic activity We were, therefore, confident that the lack of enzymatic activity at the hair cell–chalice junction did not depend on an inadequate penetration of the medium.

The enzymatic activity was observed on the plasma membrane of the efferent nerve endings at the base of hair cells type II The afferent nerve endings did not show the reaction product. In fact the gap between them and the plasma membrane of the hair cells type II was generally free of precipitate. Sometimes, only a few granules could be seen in this gap The subsynaptic cistern, located inside the hair cells type II close to efferent terminals did not show enzymatic activity

The intraepithelial efferent fibres constantly showed the reaction product on their outer surface. In places where these fibres enlarge they make axodendritic synaptic contacts with afferent nerve fibres (vestibular nerve dendrites) A precipitate was always evident in the cleft of these axodendritic synapses (Fig. 2 *a*)

Below the basement membrane the reaction

product was localized on the non-myelinated fibres (Fig. 3). In the examined areas all these fibres were positive.

The material incubated without substrate did not show any specific precipitate. The reaction occurred at all the above-mentioned sites in the presence of iso-OMPA. No reaction was obtained when BW 284 was added to the incubation medium.

DISCUSSION

The preservation of the vestibular sensory areas was not always satisfactory. A remarkable shrinkage was often observed in the cytoplasm of the sensory cells whereas the nerve chalices were swollen. The best results were obtained in the material fixed in formaldehyde and incubated in toto.

The preservation of the tissues was very poor in the frozen and thawed specimens and in those sectioned using the freezing microtome. Breakdown of membranes, cytoplasmic retraction, vacuoles and mitochondrial alterations were frequently observed in all the tissues which were badly disrupted. Greater alterations were noted in the sensory cells type I and in the nerve chalices which were quite often expanded. However, the localization of enzymatic activity did not appear changed, neither could different localizations be identified from those previously described.

The controls we made have shown that the reaction depended on enzymatic activity and not on the activity of non-specific reducing groups. The controls with inhibitors indicated that the enzymatic activity was due to AChE.

Like in the organ of Corti (Iurato et al., 1970) in the vestibular sensory areas AChE activity seems to be an exclusive property of efferent fibres, efferent nerve endings and synaptic enlargements along efferent presynaptic fibres.

On the basis of their AChE activity Dohlman et al. (1958), Dohlman (1960) and Rossi & Cortesina (1962) considered the efferent vestibular fibres as cholinergic. However, the histochemical localization of AChE in an axon does not automatically identify that axon as cholinergic in the accepted sense (discussion in Robinson & Bell, 1967). As pointed out by Eccles (1964) several other criteria have to be fulfilled before one can have full confidence in identifying a substance as the synaptic transmitter of a system of nerve fibres. Particularly important are e.g., the neuropharmacological tests. Unfortunately, in the case of the efferent vestibular system, the results of the neuropharmacological tests to-date are incomplete.

Rossi et al. (1964 *a*) found that in the rabbit the intracarotid injection of an inhibitor of AChE activity like DFP or the injection into the endolymph of the same substance (Rossi et al., 1964 *b*) caused the disappearance of AChE positivity in the efferent vestibular fibres and cristae ampullares at the side of injection. The pathogenesis of the vestibular syndrome elicited by DFP seems, at least in part, related to a reduction of AChE and a subsequent increase of ACh. It is evident that ACh and AChE play a functional role in the vestibular sensory areas, but further investigations are necessary to clarify if ACh is the actual transmitter of the efferent vestibular fibres.

RESUMÉ

La localisation de l'activité de l'AChE été étudiée dans les crêtes ampullaires et l'utricule de l'oreille interne du chinchilla, à l'aide du microscope électronique et par la méthode de Karnovsky. La réaction été positive sur la membrane plasmatique des fibres et terminaisons nerveuses efférentes. Le produit de réaction rempli l'intervalle synaptique entre ces terminaisons et les dendrites et calices des nerfs afférents. Aucun produit de réaction été observé à la jonction entre cellules ciliées et terminaisons et calices des nerfs afférents. Les contrôles ont montré que la réaction dépendait d'une activité enzymatique due à l'AChE.

ZUSAMMENFASSUNG

Die Lokalisation der AChE-Aktivität wurde in den Cristae ampullares und in dem Utriculus des Chinchilla Innenohres nach der Methode Karnovskys elektronenmikroskopisch untersucht. Die Reaktion war positiv auf dem Plasmalemm und den Endigun-

gen der efferenten Nervenfasern. Das Reaktionsendprodukt füllte die synaptischen Spalträume zwischen diesen Endigungen und den afferenten Dendriten bzw den Kelchen. Kein Reaktionsendprodukt wurde zwischen Haarzellen und afferenten Nervenendungen bzw Kelchen gefunden. Die Kontrollen zeigten, daß die Reaktion durch die AChE-Aktivität verursacht wird.

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DISCUSSION

Cath. Smith. In Mr Iurato s studies of the vestibular efferents he said that he found the reaction precipitate about small non-myelinated nerves below the basement membrane, but not related to the large myelinated nerve fibers. Does he think only the non-myelinated nerve fibers are efferent? Or could it be that the reaction product simply could not penetrate the myelin sheaths? In some studies that Dr Rasmussen and I made in the chinchilla, we found that when the efferent nerve fibers (along with the vestibular nerve root) to the vestibule were cut in the brain-stem there was evidence for degeneration in some large myelinated nerve fibers close to the basement membrane beneath the maculae.

H S Spoendlin. I was very pleased to see how much the results of Mr Iurato histochemical studies are in accordance with our degeneration studies, which indicate that all upper tunnel radial fibers are efferent fibers. Since we have seen that acetylcholinesterase is present along and around all efferent fibers and has a tendency for considerable diffusion, I think it is hardly possible to determine the sites of axodendritic synapsis by this histochemical method.

S Iurato (Reply) to *Miss Smith*. You are quite right in saying that the myelin sheath could represent a barrier to the penetration of the medium. In order to be certain that the absence of AChE activity in the myelinated nerve fibers located immediately below the basement membrane was not due to an inadequate penetration of the medium when the material was incubated *in toto* we made some trials on frozen and thawed material and on sections cut using the freezing microtome. By these techniques severe morphological alterations were found in the tissue. However this procedure did not produce a different localization of enzymatic activity Nevertheless, it cannot exclude the presence of AChE-positive myelinated nerve fibers in the trunk of the nerve.

To *M Spoendlin*. In the tunnel of Corti the upper radial fibers and those of the spiral tunnel bundle show heavy precipitate on their surface. The reaction end product is absent on the lower tunnel radial fibers. This should mean that the upper tunnel radial fibers are efferent, and the lower tunnel radial fibers afferent. It is an indirect confirmation of your experimental data. The second comment you made is on diffusion phenomena. In histochemistry these phenomena can certainly produce "false positive reactions as well as false negative reactions We have considered these possibilities in our research and we took precautions to reduce these inconveniences.

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THE PROTEIN PATTERN OF MIDDLE EAR EFFUSION IN SEROUS OTITIS MEDIA BEHIND AN INTACT DRUM

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Abstract. The ratio of protein to lipid in middle ear effusions facilitates better distinction between serous and glue effusions. Lipid concentration is related to the duration of the disease. The immuno-electrophoretic pattern of the proteins from the middle ear effusions solubilized by means of 0.25% glucose oxidase or diastase clearly demonstrates three types of protein patterns different in their gammaglobulin types and levels. There is no correlation with the plasma protein pattern, with the macroscopical aspect of the middle ear effusion nor the duration of the disease.

Different values of protein concentration in the middle ear effusion of so-called serous otitis media have been reported (Schandler 1932 Hoople, 1950· Tremble, 1951 Suehs, 1952) and ascribed to differences in water content in transudate and exudate (Ivstam, 1954 Carlson & Lökk, 1955) Protein increase was related to the intensity of the inflammatory process (Siirala & Vuori, 1954) Some authors observed that protein content increased when the effusion remained in the middle ear for a longer time (Robison & Nicholas, 1951) The albumin/globulin ratio increases in this condition, while the albumin fraction was relatively highest in the mildest inflammations (Robison, 1942 Siirala & Vuori, 1954) Electrophoretic aspects of the fluid confirmed the opinion that an inflammatory process causes local accumulation of globulins in the middle ear in comparison with the serum pattern. From *paper electrophoretic* study it was concluded that the protein composition of the effusion follows serum composition (Carlson & Lökk, 1955) with some differences between very short and longer duration of the disease (Vuori, 1959).

Immuno-electrophoretic study however gives more information about the globulin subclasses (Matti et al., 1967) In previous investigations (Van de Calseyde et al 1969), we described three immuno-electrophoretical patterns of the proteins of the middle ear effusion in chronical cases with an intact drum. There seems to be no correlation between the three types described, the macroscopical aspect of the effusion, the age of the patient, the duration of the middle ear disease nor the protein pattern of the plasma.

In this study concerning only serous and glue ear" effusions, we were able to investigate in more detail the protein content and the protein pattern of middle ear liquids. After solubilization of the specimens with glucose oxidase or diastase, the evaluation of the protein pattern is easier Urea was not added because of a possible denaturing effect on the protein moiety The protein content and the lipid content on dry weight effusion was compared in some cases. It was our aim to establish a correlation between the protein pattern of the middle ear effusion and the duration of the ear disease.

MATERIAL AND METHODS

Excluding acute, mucopurulent and purulent cases, we considered the nature of the middle ear serous and glue effusion in 59 out-patients of the E.N T Department of St. Janshospitaal in Brugge (Table I) We had a total of 77 specimens but not all were examined for all

Table I *Clinical data*

	Patients			Age			Duration of disease		
Material	Total	♂	♀	<10	10-20	>20	Specimens	<6 months	>6 months
"Glue ear"	20	12	8	15	4	1	25	7	18
Serous eff.	39	20	19	15	10	14	52	19	33
Total	59	32	27	30	14	15	77	26	51

components. Distinction was made between cases with an otological condition of long standing (>6 months) and other relatively recent cases (<6 months)

The tympanic membrane was punctured and the middle ear effusion was aspirated according to the usual operating procedures. Macroscopically blood contaminated specimens were not taken into account. We considered as "serous" specimens the citrin and non-adherent effusions and as "glue" ear specimens turbid, very thick, very adhesive, brown or in some cases, chocolate coloured effusions.

In 5 patients with glue ear and 6 patients with serous fluid, protein and lipid concentration were determined. Nitrogen as a percentage of dry weight ear effusion is determined by an ... micro Kjeldahl method. The samples are oven-dried at 105 C for protein determination and at 70 C for lipid determination. To the dried sample 200 mg selenium catalyst mixture and 4 ml sulphuric acid 98% are added, and the whole mixture is destruated for 2 hours. After addition of 16 ml 40% NaOH and 10 ml H_2O NH_3 is collected in 0 01 N H_2SO_4 by steam destillation according to Parnas Wagner Unreacted H_2SO is titrated with 0 01 N NaOH against bromocresol green-methyl red. The amount of nitrogen multiplied by 6.25 gives the protein concentration. Lipids are extracted from the dried sample with chloroform/methanol 2 1 (v/v) according to the multi extraction procedure (Blaton et al., 1970) and total lipids are calculated as the sum of the individual lipid fractions.

Two-ml secretions collected in 0 4 ml Veronal buffer pH 8.1 μ 0.1 were *solubilized* with 1 ml glucose oxidase (50% pure Mann Res. Labs., Orangeburg N J) or 1 mg diastase (191 000 WDA units/9 ms Mann) and incubated at 37 C for 2 hours. Dialysed samples against Veronal buffer pH 8 1 (μ 0 1) are centrifuged at 12 000 rpm and stored at 4 C. Before analysis of the protein pattern, samples are concentrated under N_2 for 24 hours.

Immuno-electrophoresis was carried out by the slightly modified micromethod of Scheidegger on 0 8% agarose plates in Veronal buffer pH 8 1 (μ 0 1) Untreated and treated samples were tested against rabbit anti-human serum (Behring Werke, Marburg/Lahn, BRD) both undiluted and concentrated by ultrafiltration under nitrogen and compared with analogous concentrations of pooled standard plasma in order to estimate the relative concentration of immunoglobulins.

RESULTS

1 *Protein and lipid concentration*

Table II gives the percentual nitrogen, protein and lipid concentration of the middle ear sec

Table II *Percentual nitrogen protein and lipid concentration*

Sample	Patients	% N ± S.E.	prot. ± S.E.	% lipid ± S.E.	Prot./lipid
Glue	5	6 85 ± 0.67	42.82 ± 4 19	0 60 ± 0.10	71
Serous	6	5.83 ± 0.98	36.44 ± 6.12	1 41 ± 0.25	25

Table III *Ratio of total cholesterol to total lipids*

	Total lipid		
Duration	Specimens	Average mg	Tot. chol./tot. lipid
< 6 months	16	1.5	0.19
> 6 months	6	0.63	0.62

retions. A higher amount of protein (6.4%) is observed in "glue ear" together with a lower percentual lipid value.

As previously observed, the total lipid concentration decreases in function of the duration of the disease and is accompanied by a relative increase of the total cholesterol concentration (Table III).

2. *Protein pattern*

The modified procedure confirms the existence of three protein patterns in the middle ear effusion. The influence of enzymes and of concentration of the sample can be observed in Fig. 1.

After enzyme treatment clearer protein patterns were obtained and after concentration, in some cases, IgM and transferrin were demonstrated. This modification of our procedure confirms the existence of different protein patterns in the middle ear effusion. Protein patterns of "glue ear" and serous fluids are demonstrated in Fig. 2 and described in Table IV. Type I was found only in serous fluids and shows an increase of immunoglobulins IgG and IgM. The pre-albumins, beta lipoproteins, alpha glycoproteins and antitrypsin are not demonstrated. Type II includes serous fluids and "glue ear". It shows an increase in IgA and IgM; ceruloplasmin, transferrin and fibrinogen are present, but many other plasma fractions were not observed. Type III includes only serous fluids and shows only an increase of IgG globulin.

In no cases was there a correlation between the plasma protein pattern and the middle ear effusion. Influence of the disease standing on the biochemical composition of the middle ear effusion could not be demonstrated and no significant value could be calculated.

CONCLUSION

With diastase or glucose oxidase a complete solubilization of the proteins in the middle ear

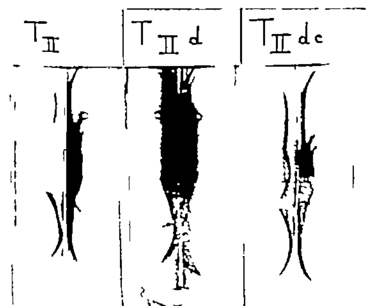


Fig. 1. Type II protein pattern of the same patient before and after treatment with diastase. *d*, diastase; *c*, concentrated.

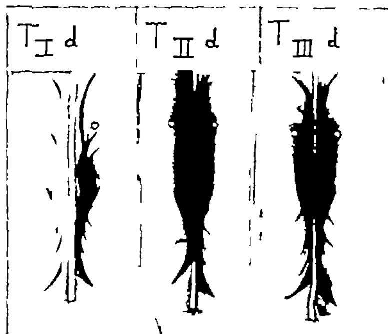


Fig. 2. The three types of protein pattern in middle ear effusion.

effusion is obtained. This improved procedure confirms the existence of three different types of protein patterns in the middle ear effusion. One of the most important findings is the increase of gammaglobulins in all types of protein patterns with modification of the gamma subclasses. This modification of the gamma globulin distribution and concentration, as was observed earlier by Vuori by paper-electrophoresis, can be ascribed to an activation of synthesis of IgG by lymphocytes or to an influence of the environment on the distribution of the gammaglobulin subclasses. We were unable to demonstrate a correlation between the plasma protein pattern, the protein pattern of the middle ear effusion, and the macroscopical aspect of the liquid. It can only be said that type II was principally observed in glue ear. A difference in the mucopolysaccharides seems to be, as known, a predominant factor in the physical aspect of the middle ear effusion, no influence being demonstrated on the protein pattern.

These results and the biochemical difference between the protein and lipid concentration in glue and serous *effusion seems to indicate* a difference in the significance of serous and glue effusion. In respect to the evaluation of the duration of the disease the study on lipids seems to be a parameter to follow by biochemical data, the duration of the disease. As discussed in an other paper (1968) the fact that some fatty acids, as palmitic acid, are badly metabolized this observation can be an inter

Table IV *The protein patterns of glue and serous otitis fluids*

Protein pattern	Number of specimens	Nature of secretion	Description
Type I	28	Serous	IgG IgM increased, α 1 glycoprotein, β LP antitrypsin, prealbumin not demonstrated
Type II	25	Serous, glue	IgA, IgM increased ceruloplasmin, transferrin and fibrinogen present, all others absent
Type III	9	Serous	IgG increased
	62		

esting information for interpretation of the prognosis of the middle ear effusions, and in this way of greater value than the immunoelectrophoretic examination. From our study on the protein pattern, lipid and fatty acid composition of the middle ear effusion, we can conclude that glue and serous fluids are different in origin, and must be considered as an exudate rather than as a transudate.

RÉSUMÉ

Le rapport lipides–protéines permet d'établir une distinction très nette entre l'otite séreuse et muqueuse. La concentration des lipides est en rapport avec la durée de l'affection. La répartition immuno-électrophorétique des protéines sol biluées par l'addition de 0,25% de glucose oxydase ou diastase, permet d'établir trois types de liquides d'oreille moyenne. Tous diffèrent du profil électrophorétique du plasma. Aucun ne présente de rapport avec l'aspect macroscopique du liquide ni avec la durée de l'affection.

ZUSAMMENFASSUNG

Die Relation Lipiden–Eiweisstoffe ermöglicht einen sehr deutlichen Unterschied zu machen zwischen seröser und mukoser Mittelohrentzündung. Die Konzentration der Lipiden hängt mit der Dauer der Krankheit zusammen. Das immunoelektrophoretisches Profil der Eiweisstoffe, aufgelöst durch Zufügung von 0.25% Glukose Oxydase oder Diastase, lässt zu in Funktion des Gammaglobulinen Types und ihrer Niveau, drei verschiedenen Typen zu unterschieden. Man findet keine Übereinstimmung mit dem elektrophoretischen Profil des Plasmas noch mit der Dauer der Krankheit.

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DISCUSSION

T Palva: I think that studies of the type Mr Calseyde showed us are of great importance. By these modern methods more information may be obtained about these obscure conditions, serous otitis media and the glue ear than about the conventional methods. I should like to ask two specific questions: did you measure the absolute protein concentrations in the middle ear fluids as compared with the 7 g% in serum, and did you equat the serum and middle ear fluid protein concentrations before doing the immunoelectrophoretic analysis If equation is not made, some of the ocular fractions may not be demonstarated at all and true comparison may not be obtained.

M Siirala: What kind of anti-sera was used Was it general antihuman rabbit serum or was it separated into anti IGA, IGM, IGG? Further were there any differences of the immunoglobulins found in different stages of the serous processes

M Escher: I it still possible, in view of the results obtained, to state that serous otitis develops in glue ear after prolonged evolution and, furthermore, that the next stage may be cholesterol granuloma?

M Zechner: Thank you for the reconfirmation of our results in the development of the cholesterol hydrops. An important rôle in all middle ear effusions is played by the mucosa itself with high enzymatic activity especially in adhesive otitis media. W could show high activity of unspecific esterases in

mucosa with large cholesterol granulomas. High alkaline phosphatase activity was also to be seen.

G Lidén. My question is a little peripheral to Mr Calseyde's paper but I should like to hear if there is any correlation between your groups and the actual tubal function in these groups.

P van de Calseyde (Reply) to Mr *Palva.* Table II gives the protein concentration of dry weight effusion In this study no systematic comparison was made with the plasma protein concentration. For immunoelectrophoresis, ear effusion was compared with analogue concentration of plasma taking into account the equation of protein concentration.

To Mr *Surjan.* No separated IGA, IGM or IGG antihuman serum was used for this investigation.

To Mr *Escher* Vous rencontrez exactement les vues que nous avions au début de ces recherches. Nous avions pensé pouvoir mettre en évidence un évolution dans le métabolisme des liquides d'otite séreuse vers la formation de glue ear" ou d'otite à cholesterine. Or dans les faits que nous avons observés, nous n avons pas retrouvé cette évolution du moins dans son aspect électrophorétique. Au point de vue clinique, il ne nous est arrivé qu'à deux reprises de retrouver plus tardivement sous forme de glue ear des cas cataloques antérieurement comme otite séreuse. Ceci sur l'ensemble des 77 prelèvements dont il est question dans cette étude. Par contre le dosage des lipides totaux nous ont donné du résultats en rapport avec la durée de l'effection, ceci aussi bien pour l'otite séreuse que pour le glue ear" Des lipides totaux diminent serais le pourcentage de cholesterol augmente dans les esters de cholesterine nous avons observé, avec la durée, une augmentation de la quantité d'acides gras saturés et particulièrement une plus grande concentration d'acide polmitique. Ceci doit retenir l'attention car ces acides sont mal métabolises et restent dans la cavité d'oreille moyenne A ce moment, ils peuvent agir comme des corps étrangers. A ce propos je voudrais rappeler les belles études experimentales de Friedmann et d'autres auteurs qui ont introduit des corps étrangers dans l'oreille moyenne et ont observé des réactions histologiques intéressantes de la paroi de l'oreille moyenne aboutissent à la formation de granulomes et notamment de granulomes à cholesterne. Il est possible que les résidus non métabolisés soient à l'origine d'une forme adhesive ou cicatricielles. Il y là une considération pathogénique à retenir

To Mr *Zechner* We have not effectuated histological investigations on the middle ear role.

To Mr *Lidén.* We have not observed statistically valuable differences on the dysfunction of the Eustachian tube between the serous and glue ear" cases.

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AUTORADIOGRAPHICAL DISTRIBUTION OF LOCALLY APPLIED DIHYDROSTREPTOMYCIN IN THE INNER EAR

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Abstract H_3-labelled dihydrostreptomycin (DHS) in isosmolar solution is perfused through the cochlea of guinea pigs. After varying incubation periods the distribution of the radioactivity in the cochlear duct is shown by autoradiography. Because of the water solubility of DHS, freeze drying of the tissue is applied. Maximum radioactivity is clearly seen over the inner and outer hair cells and over the nerve tissue of osseus spiral lamina. This seems to indicate that the specific sensitivity of the sensory cells to DHS is not only due to the long persistence of the substance in the perilymph but also to a specific affinity of the drug to their cytoplasm. Presumably DHS is attached reversibly to the ribosomes of the hair cells as described in bacteria.

The ototoxicity of dihydrostreptomycin (DHS) and streptomycin at normal dosages in animal experiments and treatment of human tuberculosis is rather low. Lehnhardt (1970) found that 4.8% of his patients had a marked hearing loss during prolonged treatment with streptomycin. Rempt (1969) found a 7.2% and Teubner (1965) a 7.3% hearing loss in their cases. If we compare these cases with those treated with Kanamycin, we find a hearing loss in 55.6% (Rempt, 1970). Considering the high number of patients which have to be treated with DHS and streptomycin, we need to know even more about the mechanism of its ototoxic effect.

The problem of inner ear sensitivity to glycosid antibiotics was clearly explained by Stupp et al. (1969, 1970) by the slow efflux of the substances out of the perilymphatic space into the vessels. But there are still two important questions to be answered.

1. Why are especially the sensory cells so sensitive to the effect of glycosid antibiotics and no other tissues of the inner ear?
2. Where does streptomycin interfere with the cell metabolism?

Several morphological observations showed that the primary effect of DHS was localized in the mitochondria (Duvall & Wersäll, 1964; Müsebeck, 1963) or in the ribosomes (Spoendlin, 1966). Our first experiments with intramuscularly-injected radioactive-labelled DHS were not successful. Radioactivity could not be demonstrated by autoradiography due to insufficient quantities present. Probably the specific activity of the substance was too low.

Therefore we administered labelled DHS locally into the cochlea to obtain higher radioactivity for autoradiography.

METHODS

Guinea pigs weighing 250–450 g were anesthetized with ether and Nembutal and the bulla tympani was opened to expose the round window. The stapes was removed from the oval window. The perilymphatic space was perfused with an isosmolar tritium-labelled DHS solution (9.8‰) diluted with the same

This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

Abbreviations BM, Basilar Membrane; DHS, Dihydrostreptomycin; IHC, Inner Hair Cells; LSO, Lamina Spiralis Ossea; OHC, Outer Hair Cell; PC, Pillar Cell; RM, Reticular Membrane; TM, Tectorial Membrane.

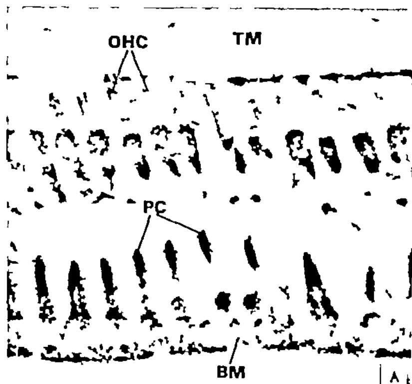


Fig 1 (A) Autoradiogram of a tangential section through one row of outer hair cells hours after injection of H_3 – *DHS*. Fixation osmic acid, glutaraldehyde. 1 100. Silver grains can be observed over the sensory cells, pillar cells and basilar *membrane* The *number of silver* grains over the nuclear region of the sensory cells seems to be slightly higher than elsewhere.

amount of Ringer solution. (Spec. act. was 0.5 Ci/g. DHS was kindly donated by Farbwerke Höchst, Frankfurt a. M) The injected radioactive amount per cochlea was about 1 mCi.

a first series the cochleae were fixed with 5% glutaraldehyde (1 hour) and 1% osmic (45 min) followed by dehydration with alcohol

In a second series the removed cochleae were freeze-dried for 48 hours immediately after dissection. All specimens were embedded in Epon 812 and sectioned on an LKB Ultrotome at 2 μ thickness for light microscopical examination. The sections were covered with Kodak AR 10 plates and exposed for 8–21 days. The resulting autoradiograms were developed with Metol and examined under a ZEISS phase-contrast microscope (Film. AGFA Isopan IFF)

RESULTS

Series 2 specimens fixed and dehydrated as described in Methods (Fig 1 A and B)

After incubation for 1 hour with radioactive DHS we saw a low density of silver grains equally distributed over the intact structures of the cochlear duct. After 2 hours several destroyed outer hair cells with clumped nuclei could be observed. Over these cells, a slight accumulation of silver grains was observed. After 4 hours, most of the outer hair cells of the organ of Corti appeared considerably damaged. The density of silver grains over the sensory cells was increased compared with the surrounding fluid space. The nuclear region particularly showed a higher activity However there was no significant difference between hair cells and other groups of the cochlear duct. Most of them showed a slightly higher radioactivity over their nuclei. Over the cells of the stria vascularis the radioactivity was significantly lower

Series 2 specimens freeze-dried (Figs 2–5)

The incubation time was 1 2 5 and 8 hours. In addition two cochleae were preincubated for 1 hour with "cold" DHS before incubation with labelled DHS.

In spite of the varying incubation times, the autoradiographical findings in all animals of

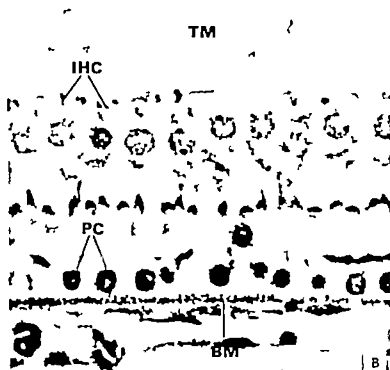


Fig 1 (B) Autoradiogram of a tangential section through one row of inner hair cells _ hours after I jection of H_3 – DHS. Fixation: see Fig. 1 (*A*). 1 100. The highest number of silver grains can be seen over the nuclei of the inner hair cells and the basilar membrane.

the second series including the preincubated animals were very similar

The unfixed and unstained tissue showed only weak contrast in the phase-contrast microscope. Though the cells were sufficiently pre served, we sometimes saw dislocation and distortion of the organ of Corti, probably due to the freezing procedure. The background of the autoradiograms was relatively high. Mor phological destruction, particularly in the outer hair cells, could be observed when the incubation time had been increased. This was in agreement with the specimens of Series 1 The autoradiograms of almost all specimens showed a significantly high number of silver grains over the inner and outer hair cells, the nerve fibers within the spiral osseous lamina, and the ganglion spirale. The ganglion cells them selves showed apparently lower radioactivity

At higher magnification the inner and outer hair cells show a striking pattern. nearly all the silver grains are located over the cytoplasm while the nuclear region rarely shows any activity at all. The statistical distribution of silver grains confirming this observation is shown in Table I.

DISCUSSION

If we compare the results of the two experimental series with locally applied DHS — one series fixed with glutaraldehyde-osmic acid and dehydrated, the other series freeze-dried — they show differing patterns of silver grains. Maximum activity after fixation and dehydration was localized at the nuclear region of the sensory cells but also of other cells of the cochlear duct, whereas the main activity of the freeze-dried specimens was clearly to be seen over the nerve tissue within the spiral osseous lamina, and the inner and outer hair cells. In this series the nuclear region of the sensory cells was nearly free from activity The different results of the two series can be explained by extraction of the water-soluble labelled DHS by the fixation and dehydration procedure. This can be avoided by freeze drying



Fig 2 Autoradiogram of a radial section through the organ of Corti incubated 5 hours with H_3–labelled DHS. Freeze-dried tissue. 700. The unfixed and unstained freeze-dried tissue gives only poor contrast. When focused on the tissue, severe damage at the outer hair cells can be observed. As the specimens are not washed out after dissection, fairly high radioactivity can be seen spread out all over the tissue. The maximum density of silver grains is clearly to be seen over inner and outer hair cells as well as over the nerve tissue.



Fig 3 Autoradiogram of a radial section through the organ of Corti incubated 1 hour with "cold" followed by 1 hour incubation with H_3–labelled DHS Freeze-dried tissue. 700. High density of silver grains over the outer hair cells.

of the tissue immediately after incubation as pointed out by Neumann (1958).

We therefore restrict our interpretation to the freeze-dried specimens, as this method seems to give the more reliable results. As mentioned above we see the maximum of the silver grains over the sensory cells and the myelinated nerve fibres of the spiral osseous lamina. Other cell groups of the cochlear duct did not show any radioactivity significantly higher than the surrounding fluid. At higher magnification we saw that the silver grains were localized mainly over the cytoplasm of the sensory cells, whereas the nuclear region was free. This observation shows that the labelled compound penetrates into the cytoplasm of the sensory cells and does not remain at the cell surface, as suggested by Duvall & Wersäll (1964).

Further we can demonstrate a selective accumulation of the ototoxic DHS in the sensory cells of the organ of Corti. As far as can be detected by light microscopy the silver grains are equally distributed over the cytoplasm of the hair cells. From our light microscopical pictures we can not yet identify to which cytostructure the DHS is bound. It is possibly attached to the ribosomal fraction of the sensory cell as described earlier by Gorini & Davies (1968) in bacteria.



Fig. 4. Autoradiogram of radial section showing an inner hair cell. For experimental conditions, see Fig. 3. ×1 100. *High radioactivity over the inner hair cell. No silver grains can be observed over the nucleus.*

This could explain the observation made by Spoendlin (1966) who described a significantly reduced number of ribosomes in the sensory cells of the vestibular end organ of the cat after chronic and acute intoxication with streptomycin. As a consequence of ribosomal intoxication or damage, we would expect an alteration in nucleic acid- and protein-metabolism. The finding of Floberg et al. (1949) who described a reduced production of ribonucleic acid in single sensory cells of the vestibular organ after streptomycin treatment confirms this assumption. Finally our experiments show another interesting fact with regards to the mode of action of the DHS on the inner ear. If we perfuse the cochlea with cold DHS before incubation with the labelled DHS we still find by autoradiography a high activity over the sensory cells and the nerve tissue, which seems to be even higher than in the cases directly incubated with labelled DHS. This seems to indicate that the unlabelled DHS molecules can rapidly be exchanged against labelled DHS molecules. Similar observations were made by McQuillen (1951) who described a reversible binding of streptomycin at the surface of *Escherichia coli* and *Staphylococcus aureus* and by Leon & Brock (1967). According to our experimental findings and the facts known from the literature, we come to the conclusion that the specific ototoxicity of DHS and probably other glycosid antibiotics is due to two different

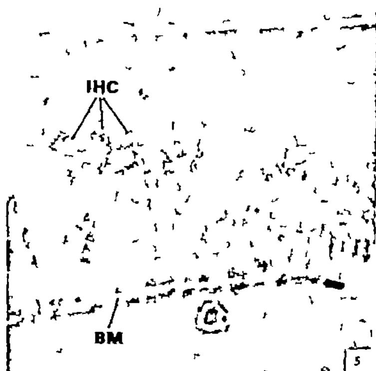


Fig 5 Autoradiogram of a tangential section through the row of inner hair cells. Experimental conditions see Fig 3 1 100. High radioactivity over the cytoplasm of the i ner hair cells. The nuclei show no silver grains.

Table 1 *Silvergrain density over different tissues of the inner ear In 20 specimens the area of about 20 20 μ was counted by a counting unit and averaged*

IHZ	OHZ	TECT M	LIMB.	NERV	STRIA.	ENDO	PERI
	53	10	10	37	15	18	18

factors (*a*) the long persistence of DHS in the perilymph as described by Stupp (1969 1970) and (*b*) a specific accumulation of DHS by the sensory cells and the nerve tissue of the organ of Corti.

Our present results obtained with locally applied DHS could not so far be confirmed by experiments with intramuscularly-injected DHS. In a recent paper Balogh et al. (1970) did not find any accumulation of intramuscularly-administered DHS in the structures of the inner ear Probably long term treatment with labelled DHS of high specific activity could give us more details on this interesting problem.

ACKNOWLEDGMENT

We wish to thank Prof. Dr J Chou, Frankfurt M., Priv Doz. Dr H. P Rohr Basel, and Mrs G Frank for their valuable assistance.

RÉSUMÉ

Pour demontrer la distribution de la Dihydrostreptomycine (DHS) dans l'oreille interne, H_3-DHS en solution isosmolaire est perfusée par la cochleé du cobaye Après des temps différents d'incubation la distribution de la radioactivité est demontré par l'autoradiographie. A cause de la solubilité dans l'eau de DHS le tissu est freeze dried" Le maximum de la radioactivité on voit clairement sur les cellules auditives externes et internes et sur le tissue nerveux de la lame spirale osseuse. Cela semble a indiquer que la sensibilité spécifique des cellules sensorielles contre la DHS dépend pas seulement de la longue

persistance du médicament dans la périlymphe mais aussi d'une affinité spécifique de la DHS leur cytoplasme. Probablement la DHS est attaché reversible ment au ribosomes des cellules auditives comme est connu chez des bactéries.

ZUSAMMENFASSUNG

Um das Verteilungsmuster für Dihydrostreptomycin (DHS) zu studieren, perfundierten wir das Innenohr von Meerschweinchen mit H_3 markiertem DHS in isotonischer Lösung. Nach verschiedenen Inkubationszeiten zeigen wir mit Hilfe der Autoradiographie die Verteilung der Radioaktivität im Innenohr. Wegen der Wasserlöslichkeit des DHS erwies sich die Gefriertrocknung der Gewebe als notwendig. Das Maximum der Radioaktivität lag eindeutig über den inneren und äusseren Haarzellen sowie dem Nervengewebe innerhalb der Lamina spiralis ossea. Die Befunde deuten darauf hin, dass die spezifische Sensibilität der Sinneszellen gegenüber DHS nicht nur auf eine längere Verweildauer der Substanz in der Perilymphe sondern auch auf eine spezifische Affinität des DHS zu deren Cytoplasma zurückzuführen ist. Vermutlich lagert sich das DHS reversibel an die Ribosomen der Haarzellen an, wie das bei Bakterien bekannt ist.

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DISCUSSION

J. G. Hall. How were the countings performed? Were they performed microscopically one square centimeter is great space to cover if on a photograph. This is not so reliable; you have to count through several pictures to get significant answer

G. Lidén. The ototoxic effect of dihydrostreptomycin (DHS) is well known clinically. Besides the hearing loss these patients also get diminished difference limen in the Békésy audiogram. The current interpretation of the diminished difference limen seems to be lesion in the outer hair cells. You mentioned that you have found lesions both of the outer and inner hair cells and of the nerve structures. Would you care to comment further on the distribution of the DHS. Is there major localization to the outer hair cells or is DHS equally distributed over the hair cells?

C. von Ilberg (Reply) to Mr *Hall*: W counted the silver grains with mask 1 1 cm on our micrographs (enlargement 1 500).

T M *Lidén*: W found no significant difference so far between the activity seen over the inner and outer hair cells. There may be some difference in different coils of the cochlea but we have not yet evaluated different areas.

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PRIMARY STRUCTURAL CHANGES IN THE ORGAN OF CORTI AFTER ACOUSTIC OVERSTIMULATION

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Abstract. 32 guinea pigs were exposed to 100–138 dB wide band noise for 1 min to 1 hour and sacrificed immediately afterwards or after short survival times. In light and electron microscopic examinations the initial structural changes proved to be: distortion of outer hair cells, buckling of sensory hairs, swelling of the dendrites to the inner hair cells, and increase of density in efferent nerve endings at the outer hair cells, followed by disintegration of hair cells and rupture of dendrites with incipient retrograde nerve degeneration at higher exposures. There appears to be a critical intensity for these initial structural changes, which only partly can be explained on the basis of a purely mechanical effect. Swelling of dendrites seems to be reversible to a certain extent, whereas the buckling of sensory hairs of the inner hair cells remains even after longer survival times.

Most of the earlier histopathological studies in acoustic trauma were made after chronic acoustic overstimulation and showed considerable survival times (for references see L. Rüedi, 1957). Only more recently the effect of jet engine-noise (Engström & Ades, 1960) and intense low frequency sound (Beagley 1965 a, b) on the ultrastructural appearance of the inner ear has been investigated. The present investigation was designed to demonstrate the immediate structural changes after relatively short exposure and it was not our purpose to study the localization and extension of damage areas, which was recently reinvestigated by Stockwell et al. (1969). We expected more information about the mechanism of acoustic damage and possibly reversible changes related to TTS.

MATERIAL AND METHODS

32 guinea pigs of 200–400 g with normal Preyer reflexes were used. Structural alterations were the main purpose of this study and the functional tests were only done with Preyer's reflex, which, however, gives fairly good information about the overall hearing function despite its high normal threshold of about 70 dB re (Rüedi, 1957).

In order to produce damaged areas in the cochlea as large as possible we used a wide band noise with a fairly even frequency representation between 500 and 10 000 Hz. For the sound exposure the animals were kept in a glass cylinder of adequate size directly attached to the loudspeaker, so that the animal was forced to face the loudspeaker. In this way we were able to produce noise intensities up to 138 dB. The animals were exposed to 100–138 dB from 1 min up to several hours duration and were sacrificed immediately following the exposure or after different survival times.

Since electron microscopic examination does not allow extensive coverage of the cochlea we confined ourselves to a study of samples of the organ of Corti from the 8–10 mm, the 14–15 mm, the 17–18 mm area and in some animals from the apical turn. The main damage area after acoustic overstimulation with high pure tones (Stockwell et al., 1969) or white noise (Rüedi, 1957) is known to be situated in the upper basal and lower second turn almost certainly including the 8–10 mm area. Thus the examined samples of the first turn are from the main damage area, the samples of the second turn from a marginal zone and the samples of the third and fourth turn most probably from outside the damage area (Fig. 1).

Every sample was used for a series of 2 μ

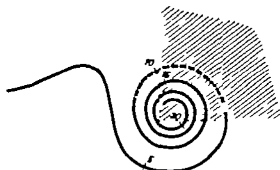


Fig. 1. Diagram of the guinea pig cochlea indicating the areas used for histological and electron microscopic evaluation (grey segment) and the main region of damage after noise exposure (interrupted line).

sections for light microscopic observation and thin electron microscopic sections of the organ of Corti. Some additional animals served for surface preparations of the organ of Corti.

RESULTS

In the evaluation of this material some precautions concerning the significance of the observed structural alterations must be observed. Many pathological alterations can occur as normal variations or spontaneous pathology in apparently healthy animals. Preparation artefacts must be excluded on the basis of a large number of normal control animals. Only regularly observed changes in the experimental animals were considered as significant. Most of them are not specific for acoustic trauma. There are great variations in susceptibility to acoustic trauma between individual animals, between the ears of one single animal and among neighbouring sensory cells.

In short exposures up to 135 dB for 1 min no significant changes could be observed, although the Preyer reflex was temporarily abolished. Stimulations with 135 dB and more for 1 min, however produced clear structural alterations in the form of distortions of outer and inner hair cells, incipient degeneration of some outer hair cells with disorder and vacuolization of endoplasmic reticulum, mitochondrial damage and sometimes rupture of the cell membrane at the cuticular pore and outflow of cytoplasmic material into the endolymphatic space (Spoendlin, 1970) Medial to its cuticular plate, the cell body of the inner hair cell bulges deep into the subtectorial space where it eventually ruptures after longer exposures (Fig. 2 B) Another striking, regularly observed feature is a buckling and confluence of sensory hairs with frequent detachment of the plasma membrane, especially pronounced at the level of the inner hair cells, where the longer stereocilia always appear to be inclined outwards. This typical cracking" of the sensory hairs is also usually seen in marginal zones (Fig. 3).

The afferent nerve fibres and endings below the inner hair cells are greatly swollen and can be blown up enormously before they rupture (Fig. 4) In contrast to the afferent dendrites associated with the inner hair cells, the efferent fibres of the inner spiral plexus and all the nerve fibres and endings to the outer hair cells remain essentially unaltered even when the hair cells are severely damaged.

The tympanic lamina appears to be shaken off the bare basilar membrane mainly in the second turn (Fig. 2 C) In some places a huge clew of dislocated tympanic lamina cells is seen attached to the basilar membrane (Fig. 2 D) The supporting structures are otherwise essentially intact except, perhaps, for a slight bending of the pillars where, however a preparation artefact cannot be excluded.

130 dB for 3 min is able to enhance sporadic degeneration of outer hair cells but otherwise causes little immediate change. By increasing the exposure time with 130 dB to 1 hour very marked structural alterations of the same type as mentioned above but more pronounced are produced. There is not only a distortion of the outer hair cells but also a general disorder of the cell contents. The normally basally located nucleus might sit in the subapical zone, the endoplasmic reticulum and the mitochondria become randomly distributed throughout the cell and Hensenbody-like accumulations of reticulum might be found anywhere in the cytoplasm, as if the cell contents had been thor

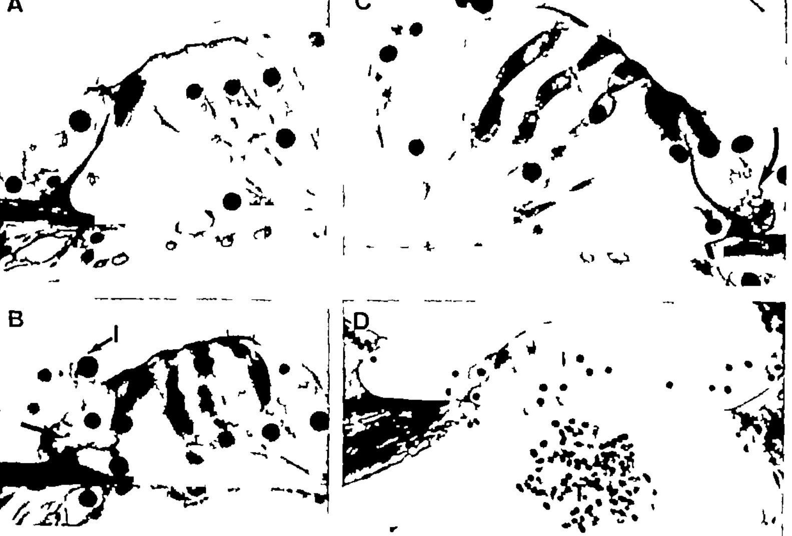


Fig 2 Light microscopic pictures of 2 μ plastic sections of the guinea pig organ of Corti: (A) Normal state basal turn. (B) Basal turn immediately after 1 min 138 dB noise e posure Note distortion of outer hair cells, protrusion of inner hair cells (*I*) int endolymphatic space and small bubbles (ollen dendrites) below inner hai cell (*arrow*). (C) Second turn, immediately aft 1 min 138 dB noise posur 1 this marginal zone f the dam ge area he first encountered lteration are: Loss of tympanic lamina buckling of the sensory hairs on the inner hair cells and swelling of dendrites below the inner hair cell (*arrow*). (D) Second turn, 1 d y fter 1 hour 130 dB noise exposure. Big lew f accum lation of tympa ic lamina cells (*T*) below the basilar membrane which otherwise is ba e of tympanic lamina cells. Loss of the m jority f outer hai cell with the supporting framework still intact.



Fig. 3. Inner hair cell from the marginal zone 3 days after 1 hour 130 dB noise exposure. The hair cell is generally unchanged, but the sensory hairs are typically inclined outwards and several hairs cluster together. This early damage seems to be essentially irreversible.

oughly shaken and mixed (Fig. 5). A greater number of sensory cells are in complete disintegration. The dendrites to the inner hair cells are not only swollen but also frequently ruptured with commencing retrograde neural degeneration and are still clearly swollen in less affected marginal zones.

In this group we found also an increased density of the efferent nerve endings at the outer hair cells caused by more densely packed synaptic vesicles and a denser ground substance between the vesicles. Taking into account the thickness of the sections and the variation of stain already existing in normal animals, great variations in vesicle density can normally be observed, but in these acoustically overstimulated animals dark and loaded nerve endings were especially striking (Figs. 5, 6).

After a survival of 4 days the organ of Corti in the main response area is, in most cases, entirely collapsed with only remnants of outer hair cells, which are expelled from the surface of the organ into the endolymphatic space. In the course of this process of expulsion of a degenerated hair cell there is an obvious tendency of the supporting structures to close the

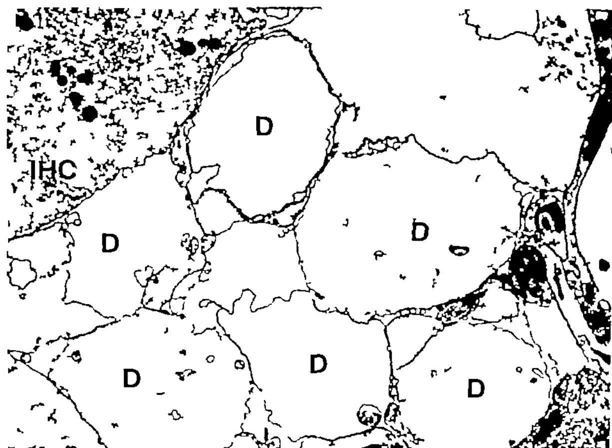


Fig 4 Area below inner hair cell in marginal zone immediately after 1 hour 130 dB noise exposure. The afferent dendrites (*D*) to the inner hair cells are blown up balloon-like The efferent inner spiral fibres (lower right angle) and the inner hair cells (*IHC*) are essentially unchanged.

p towards the endolymphatic space as soon as possible. Long before its expulsion, the damaged cell is tightly surrounded by supporting cell processes, thus preventing any leakage of endolymph into the organ of Corti through the altered cell (Spoendlin, 1970) In marginal zones the distortion of the outer hair cells and the hairs at the inner hair cells remains. There is a high incidence of myeline figures inside the efferent nerve endings of the outer hair cells, similar to the findings in nerve degeneration (Spoendlin 1969 1970) On places where the outer hair cells have completely disappeared but the inner hair cells with their associated dendrites remain no retrograde nerve degeneration occurs.

If the exposure intensity is further reduced to 125 dB only outer hair cell distortions, increased density of efferent nerve endings and dendrite swellings below the inner hair cells as well as sporadic hair cell degeneration can be found. Permanent damage after longer survival times is rare

At 120 dB significant changes are no longer observed even after exposures of several hours. Exposures up to 24 hours with 100 dB caused a complete loss of the Preyer reflex but still no detectable ultrastructural changes within the organ of Corti.

In all cases, except those with 130 dB for 1 hour the Preyer reflex recovered at least partially over a period of some days. No definite relation seems to exist between degree of damage and initial hearing loss. After 4 days, however the functional impairment seems to be related to the extent of structural damage (Fig. 7)

For more complete documentation with elec



Fig. 5. Survey of 3 outer hair cells from the main damage area immediately after 1 hour 130 dB noise exposure. Great distortion of the hair cells and disorder of the cytoplasmic elements. Very dark efferent terminals (*NE*). Pathologic sensory hair in the hair cell of 1st and 2nd row.

tron micrographs the reader is referred to an atlas on ultrastructural pathology of the ear (Spoendlin, 1970).

DISCUSSION

It has always been a matter for discussion whether damage of the organ of Corti by over stimulation is due to a direct mechanical effect of the sound wave or whether it is a consequence of metabolic exhaustion.

Short exposures with 130 dB produced a marked TTS for several hours with almost complete functional recovery but almost no detectable morphological changes (Fig. 7). This confirms our earlier findings (Spoendlin 1962) that temporary hearing loss after acoustic stimulation has not necessarily a detectable structural substrate except perhaps the changes in the nuclei of the outer hair cells as observed by Beck et al. (1956, 1960) and the swelling of dendrites to inner hair cells.

By doubling the exposure intensity from 130 to 136 dB marked structural alterations do appear followed by permanent hearing loss. Some of them have also been described by Beagley (1965 *a*, *b*). One would be inclined to consider structural alterations after such a short stimulation as a direct mechanical effect, but it must be borne in mind that even with

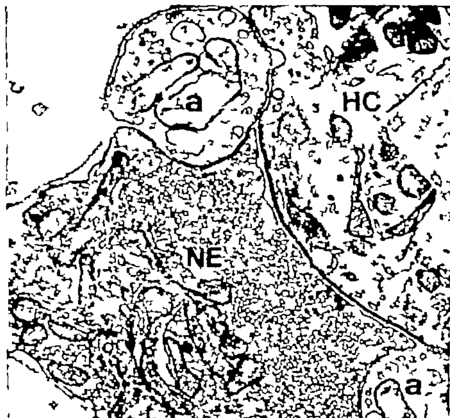


Fig 6. Efferent nerve ending (*NE*) at the base of an outer hair cell (*HC*) from the marginal zone immediately after 1 hour 130 dB noise exposure. The synaptic vesicles are very densely packed and the ground substance between them appears darker than normally. The afferent nerve endings (*a*) are essentially unchanged.

such high stimulus intensities the amplitude of movement of the basilar membrane is extreme small, within the order of Å. A number of ctural changes can hardly be explained on purely mechanical basis such as the swelling of dendrites and selective damage to hair cells within an intact, rigid, supporting framework.

After 1 hour exposures to 130 dB the outer hair cells can be entirely disintegrated whilst the supporting cells remain unchanged.

The bulging of the inner hair cells, on the other hand, might be due to the lack of solid supporting structures at this site, which seems to be a weak point. Also, the loss of the tym-

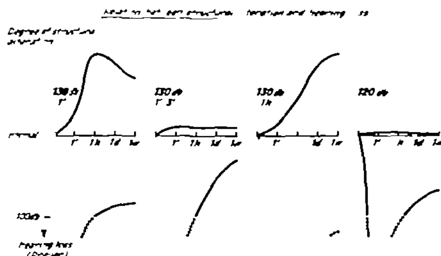


Fig 7. Diagram showing the evolution of structural and functional changes after different noise exposures.

Fig. 8. Table showing the degree of structural alteration after different survival times. Each square represents one animal.

panic lamina in the second cochlear turn is most likely a mechanical effect. These loosely attached cells seem to be shaken off the basilar membrane. The greater resistance of the tympanic lamina in the basal turn might be due to its tighter attachment and the smaller amplitude of the basilar membrane movement.

The buckling of sensory hairs would also seem to be a direct mechanical effect. Other changes of the hairs, such as confluence of several hairs or detachment of the plasma membrane, which occur even after short exposures, might equally well be metabolic effects, since they occur in the same way in toxic damage of the ear (Duvall & Wersäll, 1964). These hair alterations have a poor tendency to recover. Even 1 week after exposure, the hairs are still in the same inclined position (Fig. 8).

An interesting, hitherto unknown phenomenon is the swelling of the afferent dendrites to the inner hair cells. These blown-up dendrites have been interpreted by earlier investigators as swollen supporting cells (Beagley 1965 *b*). The demonstration of synaptic complexes with the inner hair cell and their direct connection with nerve fibres, however, allows us to identify them correctly. Exactly the same alterations are found after severe ischemia of the inner ear (Spoendlin, 1969). They are the consequence of a disturbance of membrane permeability, which in both instances might be due to a lack of oxygen or other metabolic disturbance. In any case, these dendrites appear to be a most sensitive structure to different kinds of influence.

The increase of density in the nerve endings together with the appearance of myeline figures after a period of latency might indicate that the nerve endings are activated during acoustic stimulation and eventually overstressed in overstimulation so that they show mitochondrial damage after a certain latency as a sign of metabolic exhaustion. The increased density is not seen immediately after short exposures but only after exposures of 1 hour, which would indicate that this phenomenon needs some time to build up. The assumption that the efferent innervation might have some protective function has been proposed by other authors in the desperate aim to assign some function to the efferent nerve supply of the cochlea (Davis, 1968; Johnstone, 1968).

The intensity dependence varies slightly for the different structural alterations (Fig. 9). After exposure to 120 dB for 1 hour, changes other than increased density in efferent nerve endings occur only very rarely, whereas 130 dB produces drastic immediate damage with permanent hearing loss. In short exposures up to 3 min, however, the critical intensity required to produce these structural alterations is twice as high. For stimulus intensities above

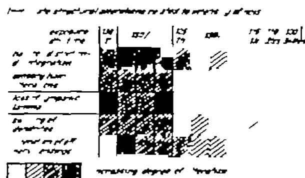


Fig 9 Table showing the degree of structural alteration immediately after different noise exposures. Each square represents one animal.

120 dB the duration of exposure appears to be very important for the extent of damage (cp. Beck, 1960) Below this critical intensity structural changes are extremely rare

The time course of the described structural changes reveals some interesting features (Fig. 8) The first changes to appear in less affected marginal zones are increased sporadic degeneration and slight distortion of the outer hair cells, swelling of dendrites and buckling of hairs of otherwise still normal looking inner hair cells. Disintegration of hair cells increases with time after the exposure, ending with the tire disappearance of the organ of Corti dur g the course of several weeks. The distortion of hair cells and sensory hairs in areas of milder damage remains constant over the entire observation period.

The dendrite swellings in marginal zones show a different evolution in time. Immediately after an adequate exposure they are a constant finding, but after survival times of more than 1 day they are almost always absent. This indicates that dendrite swellings are reversible to a certain extent, unless they rupture and degenerate

A typical delayed phenomenon is the retrograde nerve degeneration which was thought to occur only when the inner hair cells are absent. It is, however more likely that this degeneration depends upon the relationship of the dendrites to the inner hair cells which represent about 90% of all cochlear neurons (Spoendlin 1969) When these dendrites are irreversibly damaged retrograde degeneration of the majority of cochlear neurons is initiated independent of the presence or absence of inner hair cells. There are places with incipient nerve degeneration where the inner hair cells are still present.

RÉSUMÉ

Un nombre de cobayes a été exposé au bruit blanc de 110–135 db pendant une minute jusqu'à plusieures heures. Les animaux étaient sacrifiés immédiatement après l'exposition acoustique ou après un délai d'une heure jusqu'à dix semaines. A l'examen Electromicroscopique on trouvait au niveau des cell les sensorielles et des fibres nerveuses des altérations ultrastructurelles typiques qui survenaient d'une partie immédiatement et d'une autre partie seulement après un certain délai. Il y a évidence en même temps pour un dommage méchanique directe et pour une décompensation métabolique.

ZUSAMMENFASSUNG

Meerschweinchen wurden mit 110–135 dB weissem Rauschen von einer Minute bis mehrerer Stunden Dauer beschallt. Die Schnecken der Tiere wurden entweder sofort oder nach einer Latenz von einer Stunde bis zu 10 Wochen fixiert und elektronenmikroskopisch untersucht. Es fanden sich typische ultrastrukturelle Veränderungen in den Sinneszellen und den Nervenfasern, zum Teil sofort nach der Beschallung und zu einem anderen Teil erst nach einer gewissen Latenz. Es bestehen Anzeichen sowohl für eine direkte mechanische Schädigung als auch eine metabolische Dekompensation.

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DISCUSSION

J Hall. Where and how was the sound intensity measured? Furthermore did you follow the degenerating neurones centrally to see what was happening in the spiral ganglion and perhaps even further centrally?

M Lawrence As M Spoendlin has indicated, decrements in auditory function can be produced by relatively lower levels of sound without producing observable structural damage. This relates to the problem of how in the human being subject to repeated noise exposures in industry that produce each time temporary hearing loss, over a number of years permanent hearing loss results. We have reported in an industrial hygiene journal experiments in which a temporary hearing loss, determined by electrical measures, was produced in animals that were sacrificed immediately after exposure. Surface preparation revealed an occlusion of the spiral vessels of the basilar membrane and in some places of the spiral ligament and stria vascularis. Is it not possible to examine these vessels by a different orientation of the preparation so the capillary walls and possible changes there can be determined? It appears quite possible that temporary threshold shifts can be produced as a result of vascular changes interfering with the function of the sensory epithelium but without producing observable structural changes in the hair cells.

S Iurato I would like to comment briefly one point of the presentation, the swelling of the dendrites below the inner hair cells. These dendrites seem to be a very delicate structure. If I have understood, the dendrites destined to the outer hair cells are not affected by the acoustic trauma. On the other hand the swelling of the dendrites destined to the inner hair cells cannot be considered specific for the acoustic trauma, because the same phenomenon can be observed in other experimental conditions. For instance Catherine Smith and I observed the swelling of these dendrites in some animals after sectioning of the crossed OCB fibers in the floor of the fourth ventricle.

K H Vosteen. Die Veränderungen an Sinneszellen und Stützzellen nach Beschallung mit 140 dB erinnern an frühere Versuche, bei denen Endolymphe und Perilymphe vermischt wurden. Haben Sie Zerreissungen von Zellverbindungen (Hensenzellen, membrana reticularis) gesehen, die ein Eindringen von Endolymphe in das Corti-Organ ermöglichen konnten

R Hinchcliffe I have 3 questions arising from 3 comments:

(1) The validity and sensitivity of the Preyer reflex as a measure of auditory function has been questioned. Would M Spoendlin have thought it better for this study to have employed an alternative method for assessing auditory function such as behaviourally determined auditory thresholds for pure tones as a function of frequency?

(2) Stemming from other comments, were there direct or inverse correlations obtaining between the various types of structural damage?

(3) Data available from animal studies, including those conducted by my colleague Dr Sokolovski do not seem to provide support for the equal-energy hypothesis. Although the data given by M Spoendlin might be inadequate to produce statistically significant conclusions I wonder whether he has looked at the trading relationship between SPL and time, as regards production of a given amount of structural damage?

A Montandon Lors d'une récente enquête dans l'industrie en Suisse, 2 populations ont été différenciées l'une soumise à la loi habituelle de la relation de la surdité avec la durée et l'intensité, l'autre qui reste indemne Peut-on expliquer cette différence d'évolution

1°) en fixant le niveau sonore traumatisant mécanique?

2°) en précisant la nature des troubles métaboliques incriminés?

G von Schulthess The vascular aspect of the inner ear damage due to noise exposure has been neglected hitherto. As we know that there are general neurovascular reactions to acoustic stimulation it seems rather likely that these play a certain role during metabolic exhaustion. Is it even possible that there is a link to the so-called acoustic accident to sudden deafness?

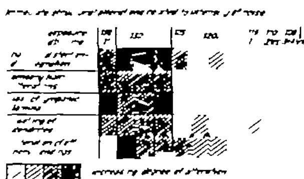


Fig 9 Table showing the degree of structural alteration immediately after different noise exposures. Each square represents one animal.

120 dB the duration of exposure appears to be very important for the extent of damage (cp Beck, 1960) Below this critical intensity structural changes are extremely rare

The time course of the described structural changes reveals some interesting features (Fig. 8) The first changes to appear in less affected marginal zones are increased sporadic degeneration and slight distortion of the outer hair cells, swelling of dendrites and buckling of hairs of otherwise still normal looking inner hair cells. Disintegration of hair cells increases with time after the exposure, ending with the tire disappearance of the organ of Corti dur g the course of several weeks. The distortion of hair cells and sensory hairs in areas of milder damage remains constant over the entire observation period

The dendrite swellings in marginal zones show a different evolution in time Immediately after an adequate exposure they are a constant finding, but after survival times of more than 1 day they are almost always absent. This indicates that dendrite swellings are reversible to a certain extent, unless they rupture and degenerate

A typical delayed phenomenon is the retrograde nerve degeneration which was thought to occur only when the inner hair cells are absent. It is, however more likely that this degeneration depends upon the relationship of the dendrites to the inner hair cells which represent about 90% of all cochlear neurons (Spoendlin, 1969) When these dendrites are irreversibly damaged, retrograde degeneration of the majority of cochlear neurons is initiated independent of the presence or absence of inner hair cells. There are places with incipient nerve degeneration where the inner hair cells are still present.

RESUME

Un nombre de cobayes a été e posé au bruit blanc de 110–135 db pendant une minute jusqu'à plusieurs heures. Les animaux étaient sacrifiés immédiatement après l'exposition acoustique ou après un délai d'une heure jusqu'à dix semaines. A l'examen Electro-microscopique on trouvait au niveau des cellules sensorielles et des fibres nerveuses des altérations ultrastructurelles typiques qui survenaient d'une partie immédiatement et d'une autre partie seulement après un certain délai. Il y a évidence en même temps pour un dommage méchanique directe et pour une décompensation métabolique.

ZUSAMMENFASSUNG

Meerschweinchen wurden mit 110–135 dB weissem Rauschen on einer Minute bis mehrerer Stunden Dauer beschallt. Die Schnecken der Tiere wurden entweder sofort oder nach einer Latenz von einer Stunde bis zu 10 Wochen fixiert und elektronenmikroskopisch untersucht. Es fanden sich typische ultrastrukturelle Veränderungen in den Sinneszellen und den Nervenfasern, zum Teil sofort nach der Beschallung und zu einem anderen Teil erst nach einer gewissen Latenz. Es bestehen Anzeichen sowohl für eine direkte mechanische Schädigung als auch eine metabolische Dekompensation.

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THE TYMPANIC MEMBRANE AS A PART OF THE MIDDLE EAR TRANSFORMER

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Abstract. Time-averaged holography an optical interference method, allowed us to obtain, for the first time, complete displacement patterns of the tympanic membrane of cat and man in response to sound. The findings are compatible with the concept of the mechanism of curved membranes originally proposed by Helmholtz (1868), which implies that an important part of the transformer mechanism of the middle ear resides in the tympanic membrane itself.

Recently we adapted in our laboratory a method known as *time-averaged holography* (Powell & Stetson, 1965) for recording the vibration pattern of the tympanic membrane

Holography in its present established form (Leith & Upatnieks, 1960) employs coherent light to record the image of an object in a phase-coded manner Two laser beams are derived from the same source. One, reflected diffusely from the object under study and the other a direct ("reference") beam, together form multiple interferences over the face of a photographic plate. After the hologram is recorded and processed, it acts as a diffraction grating when it is viewed in laser light (usually that of the reference beam), the image of the original object may be reconstructed in a true three-dimensional manner The latter is made possible by the fact that, due to the diffused reflection from the object, each point on the plate contains different information. (The term hologram was coined because the three-dimensional image was considered to contain the *whole* information about the object.)

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If the object is vibrating sinusoidally while the plate is being exposed, a *time-averaged* hologram is obtained. It may be considered to consist of a series of holograms, one for each position of the vibrating object. As it were, all these different holograms may be thought to be simultaneously present at reconstruction. However the intensity of each of them depends upon the time the object had actually spent at each position, and, during sinusoidal vibrations, most time is spent at and around the two *points of maximal displacement which are thus* being emphasized. Depending upon the relative phase between the positive and negative displacements of a given point on the object (and that in turn depends upon the displacement amplitude) their reconstructions interfere with one another hence, the image of the object is superimposed by fringes. The latter are alternatingly dark (destructive interference) and bright (constructive interference) Stationary points remain bright. The fringes represent *iso-amplitude* contours like those on geodesic maps. With helium-neon lasers, as used by us, the first dark fringe represents an amplitude of 1.92×10^{-5} cm a submicroscopic value. This calibration is rather precise, since it depends upon the wavelength of the laserlight, 623 Å in the present case, and upon the angle of illumination and observation with respect to the direction of vibration The relationship between higher-order fringes and displacement amplitude is given by a zero-order Bessel func

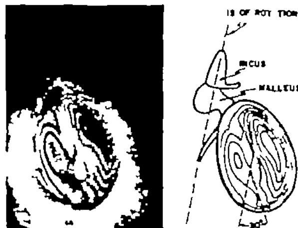


Fig 1 Left. Photograph of a time-averaged hologram of a cat's left tympanic membrane: frequency and SPL as indicated. The coarse grain ("speckle") of the photograph is characteristic of holography t the present state of the art. *Right.* Schematic drawing of the same event. The first dark fringe on the outside could no longer be discerned at this level. Therefore, the first one seen is actually the second. Thus, at the tip of the malleus is the fifth bright fringe. Translated into amplitudes, the posterior peak represents 14.6 10⁻⁶ cm, the anterior one 7.52 10⁶ cm, and that at the malleus tip 6.71 10⁶ cm. Note the inclination of the manubrial axis with respect to the ossicular axis of rotation, which is typical of most animals, but not of man. (From Tonndorf & Khanna, *Ann Otol* Aug. 1970)

once more allowing precise determina

In our hands, the resolution of small ...ces, e.g., of the size of the tympanic membrane is such that a range of 20 to 30 dB may be covered from the point of appearance of the first fringe. Although, in our experiments, the tympanic membrane was exteriorized as far as possible we found it advisable, for each given frequency to take a series of holograms at 2 dB intervals, usually over a range of 20 dB or more Only when viewing such an entire series in succession, can one be sure which fringe number should be assigned to a given dark or bright fringe on a given holographic picture.

In its present form the method of time averaged holography is incapable of giving detailed phase information. However some information about phase opposition in various sections of a given membrane can usually be derived for instance those that are seen in the various, classical vibratory modes as described by Lord Rayleigh in 1877

The actual experiments were conducted in cats. At first, fresh cadaver heads were used. However the animal was not killed until the entire preparation was completed, i.e., shortly before the holograms were being taken. In a later series, *living* animals were employed Except for a slight deterioration in picture quality in this second series, there was no difference noted between the two of them For test frequencies between 300 and 6 000 Hz, sound pressures between 90 dB and 140 dB were used to cover the frequency response curve of the tympanic membrane (TM) and to give a range of 20 dB and more for each test frequency An open sound system was used, and SPL s were monitored by a probe microphone situated at the edge of the TM. Since holography requires the object to have an opaque dull-reflecting, surface the TM was covered by an extremely thin layer of bronze powder with a particle size of 1 μ Appropriate cochlear microphonic experiments had indicated that neither the exteriorization of the TM nor its "loading" by the bronze powder had a deleterious effect upon sound transmission through the middle ear

Fig. 1 shows the photograph of a hologram of the left TM for a low-frequency signal, but at an amplitude at which an appreciable number of fringes was visible In this particular case, the first fringe had appeared at 89 dB SPL. It is no longer discernible at the amplitude of Fig. 1 the equivalent of 111 dB SPL. The drawing on the right shows that each fringe represents a continuous line The outer ones (low numbered fringes) are running around the entire membrane each crossing over the malleus in a line parallel to the axis of ossicular rotation. (In the cat, the latter forms an angle of approximately 30 with the manubrium) The higher numbered fringes however indicate the formation of two "mounds" i.e., limited areas of maximal displacement a lower one anteriorly and a higher one posteriorly The manubrium, even at its

very tip, has a smaller amplitude than either of them. Ratios (posterior maximum, anterior maximum, tip of the malleus) were typically on the order of 2.2 1.06 1.0. That is to say while the manubrium mallei rotated in fact like a stiff bar around the ossicular axis as described by E. Bárány (1938), the membrane itself did not act like a stiff plate as reported by Békésy (1941).

The type of displacement shown in Fig. 1 and found in *all* of our preparations, i.e., maximal displacements in the posterior and anterior quadrants that were larger than that of the malleus, was first postulated by Helmholtz (1868). He had proceeded to show that what he called the "curved membrane principle" constituted a lever system of which the catenary is a well-known example. As shown in Fig. 2B a curved string upon which an evenly distributed force *F is acting produces two end* forces *T*. Relations between *F* and *T* are similar to those between the two forces acting upon a lever of the first class shown in Fig. 2 *A*. The role of the force arm is played by the ratio of the sag *h* to span *a* times a constant or as stated by the simplified equation below essentially by curvature *C*. In other words, the lesser the curvature the larger the force transformation from *F* into *T*. It occurs, of course at the expense of displacement amplitudes as shown in Fig. 1. To illustrate this point further if one tried to pull such a string completely straight, the forces at both ends would rise to infinity. That, of course, is impossible.

It is clear that all radial fibres around the manubrium must act as such curved strings. Simple consideration will show that all of them should be expected to contribute in about the same manner to the entire transformation process *or in other words, that their action is* integrated over the entire surface of the TM. For as the smaller leverage grows larger from the short process to the manubrial tip, the curvature of individual radial fibers increases, i.e., their leverage becomes smaller. Therefore, the product of both levers may well be constant for all points along the manubrium.

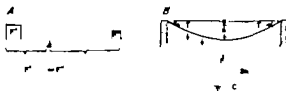


Fig. 2. (*A*) Lever of the first class and its equation. If the latter condition is met, the system may act as a *matched transformer*. (*B*) A curved (parabolic) string and its lever principle. For explanations see text. (From Tonndorf & Khanna, *Ann Otol* Aug. 1970.)

Our best current estimate of the total transformation ratio of the cat's middle ear to which the average lever ratio of the TM and that of the major ossicles as well as the area ratio of TM to stapes footplate contribute is in excellent agreement with its value as measured by us at an earlier occasion (Tonndorf et al., 1966). (This is only one of a number of considerations necessitated by Helmholtz postulate and by our own findings. Space does not allow discussion of them all here. They will be the subject of a separate paper.)

Attention was paid in our experiments to the changes in the TM's displacement pattern with increasing frequency. (Fig. 1 had shown the basic mode below the resonant point.) There were three forms of change: (1) Above 3 kHz, the two single "mounds" in the posterior and anterior sections respectively began to sub-divide into multiple mounds, their number and complexity increasing with frequency. However no signs of out-of phase motion, i.e., well defined nodal lines, as one might have expected from Lord Rayleigh's classical description of 1877 were ever found. (2) Points of maximal displacement moved toward the periphery, i.e. away from the manubrium. (3) *The volume displacement for constant SPL*, which could be calculated from photographs such as Fig. 1 was found to be independent of frequency up to 1 kHz. Thereafter it began to decrease to become once more independent of frequency above 4 kHz, the ratio between both levels being about ten. This latter value, together with a series of additional simple ex

periments, suggested that, while at low frequencies ($f < 1$ kHz) the entire TM acts as the receiver at higher frequencies ($f > 4$ kHz) this role is played by the manubrium with the TM acting merely as a baffle There is a range of transition from 1 kHz to 4 kHz between these two modes.

NOTE

The application of time-averaged holography to the recording of displacement patterns of the TM is described in much greater detail in Khanna (1970). A slightly more detailed description of the present findings is given in Tonndorf & Khanna (1970). Several more detailed papers are in preparation.

RÉSUMÉ

L'holographie est une méthode basée sur des interférences optiques. Grâce à elle nous avons pu enregistrer pour la première fois des images de l'excursion de la membrane tympanique chez le chat et chez l'homme, soumis à un stimulus sonore. Ces images confirment l'hypothèse originale de Helmholtz (1868) concernant "la mécanique des membranes courbées et démontrent qu'une partie importante des mécanismes transformatoires de l'oreille moyenne est située dans la membrane tympanique elle-même.

ZUSAMMENFASSUNG

...ie ist eine optische Interferenz Methode ... ihrer Hilfe wurden erstmalig Bilder der Auslenkung des *Trommelfells auf Schalleinwirkung* gewonnen. Sie bestätigen das ursprüngliche Konzept von Helmholtz über die „Mechanik gekrümmter Membranen (1868) und zeigen, dass ein wichtiger Teil des Transformator Mechanismus des Mittelohres direkt im Trommelfell liegt.

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DISCUSSION

H Davis: What intensity level were employed and were the movements linear at these intensities? Do the patterns of vibration change as a function of intensity or only of frequency? Please explain again how the transformer ratio from tympanic membrane to stapes is modified at high frequencies when the tympanic membrane no longer vibrates as a single stiff cone.

R Hinchcliffe: I wish to refer to the decoupling of the tympanic membrane and the ossicular chain at higher frequencies. Many mammals have their maximum sensitivity in the range where presumably the decoupling occurs. Presumably then the relative size of the manubrium will be extremely important in the various mammals. Have you looked at this?

J Tonndorf (Reply) to Mr *Davis:* The technique was mainly developed by my associate S. M. Khanna, who is an engineer by training

(1) The SPL at detection threshold ($1.2 \cdot 10^{-6}$ cm) was 85–90 dB (2) A hologram was then taken at every dB. At high intensities signs of the 2nd harmonic were often found in the pattern. However at lower intensities we are sure that distortion (optically speaking) did not play a role. (3) The transformer ratio shown in the slide applies only to low frequencies At higher frequencies it becomes smaller as the impedance increases.

To Mr *Hinchcliffe:* The area ratio manubrium to tympanic membrane in cat (≈1 10) is slightly larger than that in man.

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ANALYSE AU SPECTROSCOPE DE LA VOIX DE LA PERRUCHE

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Chez la perruche la voix se forme dans la syrinx. L analyse spectroscopique montre un spectrum des fréquences assez large. Les variations des amplitudes sont formées dans tous les bandes fréquencielles. En même temps les éléments significants pour l'intelligibilité sont les mêmes en comparaison avec la voix chez l'homme. L analyse spectroscopique de la voix de la perruche montre la possibilité de la phonation de la voix dans les mêmes bandes fréquencielles en divers systèmes de la communication.

Que l'intelligibilité du parler humain dépend de certaines régions, c est un fait bien connu. Nous savons aussi que ces régions se répètent sans égard à la langue dont un être humain se sert. J'ai essayé de constater si l'intelligibilité des sons produits par la perruche imitant le parler humain soit conditionnée par les mêmes fré quences que celle de l'homme ou par des fréquences proches de ceux de l'homme

Bien que les oiseaux possèdent un larynx au squelette cartilagneux et une rimaglotidis bien formée, ils ne se servent du larynx que pour la respiration. Les sons qu'ils émettent dans le but de communiquer ne se forment pas *dans le larynx, mais plus bas, dans l'appareil* respiratoire à l'endroit où la trachée bifurque et où se forme un plus ou moins grand nombre de plis qui flottent suivant le rythme de l'expira tion. Une série de ces plis forme un organ spécial, connu dans la zoologie sous le nom de siringa ou sirines.

La perruche possède elle aussi cet organ. Il est formé de deux plaques cartilagineuses et de deux membranes — membrane tympaniformis externe et interne — dont les vibrations for ment les sons.

Un groupe spécial de muscles provoque la tension et le relâchement des membranes. Plus la capacité de produire un registre de son est grande un plus grand nombre de muscles par ticipe à la production des sons.

La perruche réussit à changer l'échelle du ton en contractant et en étirant la trachée ces contractions et ces étirations sont rendues possibles par des intervalles cartilagineux munis de bagues cartilagneuses. Qu'il soit possible d'apprendre à la perruche de reproduire d'une ma nière parfaitement intelligible des mots isolés et même de courtes phrases est donc un fait évident et, en plus, compréhensible.

Bien qu'il soit connu que le parler en tant que moyen de communication, n est l'apanage que de l'homme on peut pourtant prouver que pour la formation des complexes phoniques correspondant au registre du parler humain, le larynx n est pas indispensable et qu'il est, chez les perruches dressées, parfaite ment substitué par le principe du fifre.

Mon but était donc d'étudier les éléments *physiques et les qualités du parler des per* ruches. J'essaierai d'expliquer quels sont, du point de vue physique, les éléments du parler des perruches, et je tacherai aussi de constater si quelque ressemblance existe parmi ces élé ments dans la composition du spectre de la voix de l'oiseau d un côté et la composition du spectre de la voix d'homme de l'autre côté.

Dans les deux cas, l'analyse au spectroscope a été faite à l'aide du système à deux dimensions notant les fréquences et l'intensité.

Pour l'analyse spectroscopique comparée, je

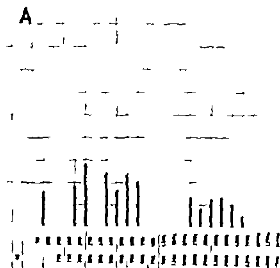


Fig 1 La oix d'homme

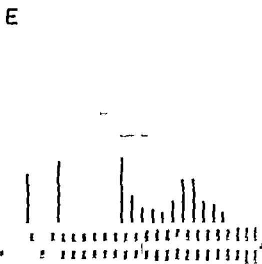


Fig 3 La voix d'homme.

me suis servi des spectrogrammes de la voix humaine normale et des spectrogrammes de la voix de la perruche

Le matériel de recherches a été minutieuse ment choisi parmi les enregistrements au ma gnétophone des voix relativement bien intelligibles de plusieurs perruches qui disposaient d un certain nombre de mots et de courtes phrases. Ensuite, de l'homme et de l'oiseau, j'ai relevé uniquement les voyelles pour les soumettre à analyse spectroscopique Je me suis décidé r les voyelles *a e I o u* Celles-ci étant les porteurs de l'intelligibilité et en même temps se prêtant le mieux pour l analyse en question.

Ensuite j'ai procédé à l analyse comparée de la voix d'homme et celle de la perruche dans le but de constater et de souligner les détails par lesquels les sons diffèrent, ou éventuellement, se ressemblent.

Analyse et comparaison du phonème a

Les deux spectrogrammes de ce son indiquent qu'il y a continuité à l'intérieur du spectre. Chez l'homme le groupe amplitudinal de la voix est accusé dans le parage de 740 à 1 600 Hertz, à l'intérieur duquel le maximum se trouve à 950 Hertz. La voix de la perruche a son groupe amplitudinal dans la région de 950 à 4 000 Hertz, à l'intérieur duquel l amplitude maximum est dans la région de 1 600 à 2 000 Hertz.

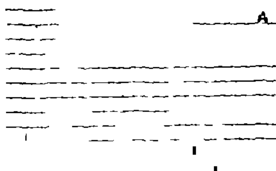


Fig La voix de la perruche.

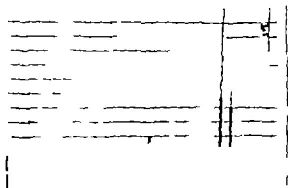
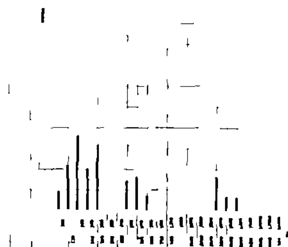


Fig 4 La voix de la perruche.



F g 5 La voix d'homme.

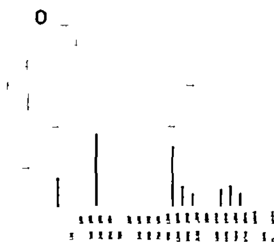


Fig 7 La voix d'homme.

Analyse et comparaison du phonème e

Ce qui est caractéristique pour ce son c est que la continuité du spectre existe si le son est formé par la voix d'homme, pendant que le son e émis par la voix de la perruche montre une discontinuité à l'intérieur du spectre. L étendu du spectre est en substance la même dans la région de 55 à 6 200 Hertz.

Le même détail pour les deux voix c est l'existence d un groupe prononcé d'amplitudes plus fortes situées dans la région des plus basses fréquences où se trouve en même temps pour les deux voix, la région de la plus grande amplitude

Analyse et comparaison du phonème i

Le spectre de la voix humaine est discontinu, pendant que celui de la perruche est continu. Le registre de la voix d'homme est entre 90 et 5 000 Hertz, pendant que l'étendue du spectre de la perruche va de 70 à 1 000 Hertz. Leur caractéristique commune est l'existence d un groupe d'amplitudes dans la région basse du registre des fréquences où se trouve aussi, pour les deux voix, la plus grande amplitude

Analyse et comparaison du phonème o

Le spectre de la voix d'homme est discontinu, celui de la voix de la perruche continu. Le registre des fréquences est presque le même pour les deux voix il va de 70 à 1 000 Hertz.



Fig 6 La voix de la perruche.

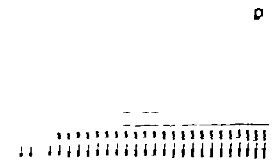


Fig 8 La voix de la perruche

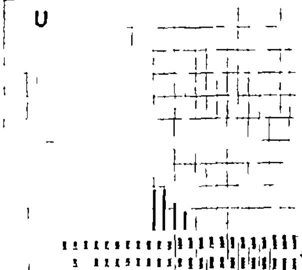


Fig 9 La voix d'homme

Le groupe à l'intérieur du spectre, situé dans la région centrale des fréquences, ou se trouve aussi la plus grande amplitude de deux voix est caractéristique pour ce son.

Analyse et comparaison du phonème u

A l'intérieur du spectre de la voix humaine il y a continuité visible, mais discontinuité chez la perruche. Le spectre de la voix d'homme se entre 70 et 1 200 Hertz, celui de la voix la perruche entre 55 et 7 800 Hertz. Les deux voix ont un groupe prononcé d amplitudes situées dan sla région de basses fréquences mais la voix de la perruche a encore un groupe spécial d'amplitudes dans la région des hautes fréquences.

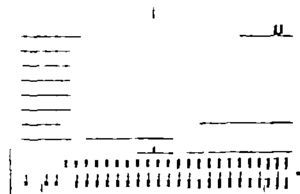


Fig 10 La voix de la perruche.

CONCLUSION

La voix de la perruche formée par un mécanisme en dehors du larynx, c est à dire par la siringa, nous surprend par la sonorité de ses éléments du parler et par l étendue de son échelle de fréquences.

L analyse au spectroscope de la voix de la perruche devoile quelques faits importants. Avant tout ce qui nous impresse c est le spectre relativement étendue de tous les sons analysés. Les variations amplitudinales sont en général bien marquées, présentées dans les régions de fréquences basses et hautes, selon la structure de chaque son.

Il est important de souligner que tous les sons analysés étaient des voyelles qui usent en plus grande quantité la force de l'air chassé et que malgré tout l'intelligibilité de tous les phonèmes analysés était relativement bonne bien que la colonne d'air ait été formée d'une restreinte quantité d air dont peut disposer la perruche Cela prouve qu'il existe un système économe pour la consommation de l'air qui rend possible d'obtenir la sonorité lors de l'emission de chaque son.

Une analyse comparée minutieusement des spectrogrammes de tous les sons présentés indique certaines ressemblances des éléments *dont dépend l intelligibilité des sons. Quant* à l'étendue des fréquences, il est important de souligner qu elle est, en principe le même dans les deux systèmes d analyses. On a l'impression que le spectre de la perruche soit plus restreint que celui de l'homme.

Les variation des amplitudes à l'intérieur du spectre sont relativement bien présentées dans les deux analyses. Les oscillations y persistent tenant compte de chaque son et de l'endroit ou il se forme.

Je souligne que presque tous les sons ont deux groupes séparés d amplitudes et que dans l'un d'eux se trouve toujours la région avec la plus grande amplitude

Par conséquent, c est le fait que dans l'analyse comparative de tous les voix présentées apparaissent des groupes fréquentes présentant

les analogies déterminées qui se trouve simultanément dans la voix analysée de l'homme et de la perruche.

Ces groupes contiennent toujours dans leur région aussi les amplitudes maximales lesquel les seraient aussi les porteurs principaux de chaque voix particulière.

En conséquence, il est tout à fait claire et analytiquement élucidé la capacité de la perruche de pouvoir imiter la parole de l'homme vu que les éléments singuliers dans l'analyse sont présentés à côté de toutes les voix dans la région fréquente identique.

SUMMARY

The voice of the parrot is formed extralaryngeally in the syrinx. Spectroscopically wide frequency range could be demonstrated. The variations of the amplitudes are formed in all frequencies. The presentation of the elements which are of significance for the intelligibility are the same as in the human voice. Spectroscopic analysis of the voice of the parrot points out the fact that in various systems of communication understandable sounds are formed in the same frequency range.

ZUSAMMENFASSUNG

Die Stimme des Papageis wird extralaryngeal in der Syrinx gebildet. Ihre Frequenzen zeigen im spektroskopischen Bild ein ziemlich breites Spektrum. Die Amplituden ändern sich in allen Frequenzen und die Elemente, von denen die Verständlichkeit abhängt, sind jenen des Menschen gleich. Die spektroskopische Analyse zeigt also dass unser Verständnis der Sprache des Papageis durch dieselben Elemente gebildet wird, von denen auch die Verständlichkeit unserer Sprache abhängt.

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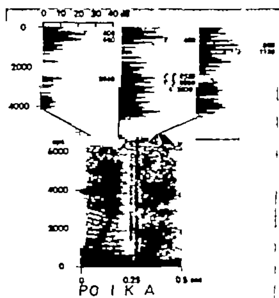
DISCUSSION

K. Sedláček. Allow me please, to comment on the very interesting paper of Mr Bauk from another point of view i.e. the physiological and communicative one. In my own paper I mention the communicative process of birds in species Agapornis roseicollis, the summary of which states that the birds are able to hear and to distinguish very precisely the absolute and relative pitch of tones of very short duration (as short as 5 msec or less). They produce very short and quick melody patterns, this melody being not perceptible for human hearing. There is also a very perfect analysis of the pitch of sounds.

Now the question arises, how it is possible that the parrots of Mr Bauk can hear differentiate and imitate not only the pitch (the melody) of speech, but also the timbre of vowels, when their labyrinth is not adapted to band-pass analysis because their lagena and basilar membrane are too short. It seems to me that the answer could lie in our results: The parrots are able to hear and differentiate the pitch of very short bursts of tones. In this way it is possible that they hear a vowel not as tone with a certain timbre (according to the place principle) but as very quick repetition of very short bursts of the formant frequency. That means that their hearing follow the very old formant theory of vowels of L. Hermann.

O. Meurman. This brings up many interesting points of the origin of the different speech elements. We have had the opportunity to analyze words spoken by magpie (Pica Pica). The picture shows spectrogram of the Finnish word "boy" (Poika). There is heavy background noise, coming from the air running through the trachea. The Finnish language is rich in diphthongs and in this picture we can see how the second formant glides from 600 cps in o to more than 3000 cps in i. In man this is done by movements of the tongue. In magpies the tongue is immobile. It is surprising to find how similar the spectrographic picture is compared with that of human voice.

V. Bauk (Reply) to M. Sedláček. Nous avons examiné la voix de l'homme en comparison avec la voix de la perroquet près l'examination de la voix esophageal. Nous avons trouvé la même spectre avec la même l'amplitude maximum.



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CHANGES IN THE TRACHEAL RESPIRATORY MUCOSA OF RATS FOLLOWING EXPERIMENTALLY INDUCED BURNS OF THE SKIN INFLUENCE OF RESERPINE

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Abstract The tracheal respiratory mucosa in rats sacrificed on the second and fourth day following experimentally induced burns of the skin was submitted to histological study Pathological changes were found in the respiratory mucosa of all animals with hepatic and kidney damage. In a second experiment the attempt was made to prevent kidney and liver lesions by administration of Reserpin The correlation between kidney and liver lesion and the changes in the respiratory mucosa are discussed.

It is a known fact that hepatic and renal damage is quite common in cases of severe and extensive skin burns (2nd and 3rd degree) In an earlier experiment on rats, Femenić was able to prove that changes of the respiratory mucosa following burns of the skin were not only due to the direct effect of hot air inspired, but that the damage probably depended greatly on pathological changes in the hepato-renal system.

In our further research work we investigated the protective effect of small doses of reserpine on the liver and kidney after the skin was exposed to burns and made a histological study of the possible changes in the respiratory mucosa of the trachea under these conditions.

We were prompted by our experimental experiences to apply reserpine in this investigation, because Krajević had demonstrated the protective effect of small doses of reserpine on the development of an experimental ulcer in the stomach of a rat. It was thus established that the protective power of reserpine was in its vasodilator effect. Clower Douglas & Carrier showed too that the toxic effect of carbon tetrachlor on the liver of a rat may be reduced by application of reserpine

MATERIAL AND METHODS

We used 60 healthy male rats weighing between 150 and 180 g. The animals were anaesthetized with urethane (2 mg/kg of body weight) The skin on the back was shaved, leaving a clean surface of about 24–30 cm^2 In 40 animals the area prepared in advance was soaked with 1.5 ml of 96% ethanol which was subsequently ignited thus inducing 2nd and 3rd degree burns. The inspiration of hot air was prevented by a collar on the neck of each animal.

Reserpine (Boehringer Mannheim) was used twice daily at 0.5 mg/kg of body weight intramuscularly in 20 of the animals which were exposed to burns on the skin. The remaining 20 animals with skin burns were used as a control group A third group of 20 animals not exposed to skin burns served as an absolute control group All three groups of animals survived under identical conditions. Food and water were taken according to need.

Ten animals of each group were sacrificed after 48 hours and the remaining animals 96



Fig. 1. Tracheal respiratory mucosa of rat 96 hours following experimentally induced burns of the skin. The protective effect of reserpine.

hours following the experimentally induced burns. The kidneys, livers and tracheas of these animals were removed and prepared for histological investigation. After fixation (5% formalin solution) the material was cut (multiple paraffin section) and routine haematoxylin and eosin stains were used.

RESULTS

In the animals sacrificed 48 hours after the experimental burns had been induced only insignificant degenerative changes in the kidneys and the liver were established. The respiratory mucosa was fully active and the respiratory epithelium intact though signs of inflammatory reaction were seen in the submucosa. These findings were within the range of the stress reaction described by Burian & Stockinger (1956).

In the animals sacrificed 96 hours after being exposed to experimental burns and subsequently treated with reserpine, hyperanemia with mild degenerative changes were seen in the kidneys and the liver. The respiratory mucosa was preserved, i.e. the epithelium was regular, cylindrical and ciliated with a blanket of mucus and dilated blood vessels with sign of mild inflammatory infiltration in the submucosa (Fig. 1).

The control group of animals, i.e. those animals with burned skin which were not given treatment, showed degenerative changes not only in the kidney and liver but in the respiratory mucosa as well. The epithelium of the respiratory mucosa was low, irregular and cilia appeared only in individual sites. The blanket of mucus was scanty. In the submucosa signs of mild inflammatory infiltration were seen (Fig. 2).

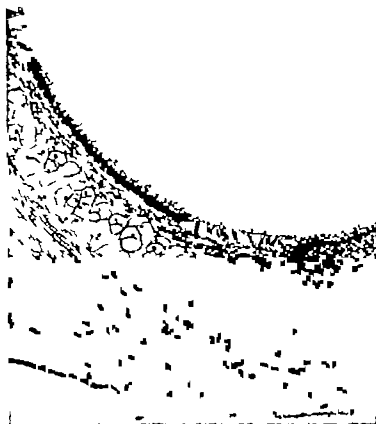


Fig. 2 Tracheal respiratory mucosa of a rat 96 hours following experimentally induced burns of the skin.

The kidney liver and respiratory mucosa in the animals not exposed to burns showed no athological changes.

DISCUSSION

Summing up the results of the experiment it is obvious that reserpine acts protectively on the kidney and the liver This protective effect of reserpine cannot be explained in our experiment. It may be based on vasodilatation, i.e better supply with blood of the parenchyma tous organs but one cannot exclude other factors, such as, e.g., its antiphlogistic effect.

With regard to the respiratory mucosa, the protective effect of reserpine is quite clear but it was impossible to discover whether the protective mechanism passes via the kidney and the liver or whether it protects the respiratory mucosa and the parenchymatous organs directly

RESUMÉ

La moqueuse respiratoire dans la trachée fut analysée histologiquement deux et quatre jours après une brûlure expérimentale de la peau. On trouvé des changements dans la moqueuse respiratoire de tous les animaux, qui valent en même temps une lesion du foie et des reins Dans un second expériment on a tenté de prevenir les lésions du foie et des reins par l'administration de Reserpin La corrélation entre les lésions du foie et des reins d'une part et des changements de la moqueuse respiratoire d' utre part est discutée.

ZUSAMMENFASSUNG

Die respiratorische Schleimhaut in der Trachea on Ratten wurde nach experimentellen Verbrennungen der Haut untersucht. Die Tiere urden m zweite und am vierten T g nach der Verbrennung geopfert Pathologische Veränderungen wurden nur bei denjenigen Tieren gefunden welche auch eine Schädigung der Leber und der Nieren zeigten. I einem zweiten Experiment wurde der Versuch gemacht mit Reserpin die Leber und Nierenschädigungen zu verhindern. Der Zusammenhang zwischen der Leber und Nierenschädigungen und der Veränderungen in der respiratorischen Schleimhaut wird besprochen

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IMMUNOLOGICAL ROLE OF HUMAN TONSILS

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Abstract. The immunological function of human tonsils was studied by tissue culture technique Micro-fragments were prepared from tonsils after tonsillectomy of officially vaccinated children. Parker 199 medium was used supplemented by plant mitogen (phytohemagglutinin). The tissue fragments were cultivated with and without pecific stimulator diphtheria toxoid. After 4 days, inoculation antibody was produced in the cultures stimulated with antigen. The diphtheria antitoxin titres were measured by hemagglutination. On the basis of comparison of human and animal experiments it was concluded that, in our experiments, human tonsils behave as a non-regional lymph node and that they have an immunological memory

Antibody production by human tonsils is a well-known problem (Corbetta, 1969 Drabe I Fioretti, 1961 Koburg, 1966 Meyer Gottesberge 1965 Naumann, 1957) This hypothesis is based first of all on results of morphological and immunomorphological investigations. During the last 10 years, modern immunology has grown into a new branch of medical science and gives us new and better possibilities to study the immunological role of lymphoid organs. The main goal of modern immunological research is to study not only the end-results of immunological processes, but is also centred on establishing the site of antibody production and the cellular basis of immunity

The immunological process at cellular level may be investigated by means of tissue culture technique. This method was used by us to prove the antibody producing capacity of human tonsils.

MATERIAL AND METHODS

Tonsils were obtained immediately following surgical excision from 4–6-year-old children who had been immunised against diphtheria, pertussis and tetanus, though naturally a booster dose was not given before the operation. The removed tonsils were minced into fragments of about 2 mm³ and 2–3 fragments were placed into a test tube containing 1 ml medium. Parker 199 medium was used containing 100 U/ml penicillin and 50 μg/ml streptomycin and supplemented by plant mitogen (phytohemagglutinin) Diphtheria toxoid (0 001 Lf/ml) was employed continuously as a specific stimulant. The fitted tubes were placed in a thermostat at 37 C We changed the medium every 24 hours during a period of 9 days.

Antibody assays Diphtheria antitoxin titres in the medium were measured by hemagglutination as published earlier by Surján & Nyerges (1968).

Tonsils of 10 children were investigated. In each case six test tubes were used containing antigen and four tubes as control without the antigen.

RESULTS

In the tissue culture medium of five tonsils, antibody was detected from the fifth day on (Fig. 1) During the first 4 days antibody was not found. The control experiments always gave negative results.

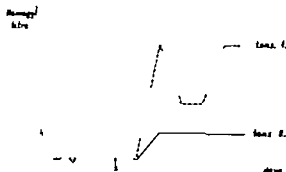


Fig. 1. In vitro antibody production of human tonsillar fragments.

In the other 5 cases of investigated tonsils the tissue cultures were infected, and the media were therefore unsuitable for hemagglutination.

These results prove that human tonsils are able to produce diphtheria antibodies in tissue culture.

DISCUSSION

For a correct interpretation of this result we must take into consideration the results of other investigations as follows:

Many animal experiments were performed by M. Surján & Surján Jr (1969). They demonstrated that lymph node fragments of rabbits hyperimmunised with tetanus toxoid continued to produce antibody in vitro for several weeks.

They compared the antibody producing capacity of regional and non-regional lymphoid organs. A booster dose was given subcutaneously 3 days before surgical excision. In these experiments the prominent role of the regional lymph node was demonstrated. These fragments are able to produce antibody in vitro without in vitro antigen stimulation, for 2 weeks. In the same condition, fragments of remote lymph nodes cease antibody production on the second day (Fig. 2 A).

The differing behaviour of regional and non-regional lymph organs is understandable on the basis of Hall's messenger lymphocyte theory (Hall et al. 1967). He found that after antigen stimulus blast type cells appear in the efferent lymph vessels of the regional lymph node. These cells might be immunological messengers and they are responsible for propagating the induction of immune response in the remote lymph organs.

The prominent role of the regional lymph node is proved by the fact that this organ enlarges twofold macroscopically on the third day and its histochemical picture shows a mass of pyroninophil plasma cells. In this stage the fragments of regional lymph node are able to synthetize antibody in vitro without further antigen stimulation. At the same time the remote organs do not have enough messenger lymphocytes: they need antigen stimulation in vitro.

After subcutaneous inoculation, the tonsils or spleen are not comparable with regional lymph nodes, because the latter have a double role: as a result of antigen stimulation they send informative cells to other lymph nodes, and at the same time they themselves produce antibody.

In these animal experiments the tonsils behave like non-regional lymph nodes. The fragments of tonsils are able to produce antibody for several weeks if they receive antigen stimulants continuously. Without in vitro stimulation tonsils and non-regional lymph nodes produce antibodies for 1–2 days only.

Until the eighth day the propagation of messenger lymphocytes reaches the remote organs too: the fragments prepared at this time continue antibody production in vitro, as does the regional lymph node. If the booster dose is given 8 days before excision, the regional and non-regional lymph nodes are similar because at this time they have received enough messages (Fig. 2 B).

Immuno-memory was also investigated in animal experiments by tissue culture technique. For this purpose lymph node fragments of rabbits, immunised 1 year previously, were cultured, but the animals did not receive a booster dose. These were the immediate previous experiments on which the study of human ton

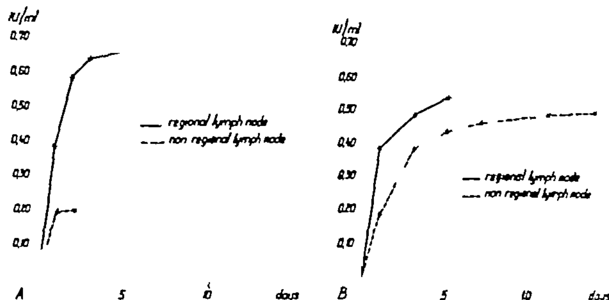


Fig. In vitro antibody production of lymph nodes of rabbits. (A) 3 days after the booster doses. (B) 8 days after the booster doses.

sils was based, because in case of human beings there is no possibility of giving booster doses before tonsillectomy. These experiments have shown that the lymphoid fragments excised 1 year after immunisation are able to produce antibody from the fifth day on, but only with continuous in vitro stimulation. The results of experiments of human and animal tonsils are similar and thus it was concluded that human tonsils also have an immuno-memory.

Apart from this tissue culture technique the antibodies (IgM, IgG) in tonsils were recently detected by fluorescent staining (Corbetta, 1969). Recently Merler & Janeway (1968) have separated fractions possessing properties of immunoglobulins from small lymphocytes of tonsils of children who had been previously immunised with diphtheria and tetanus toxoids. The antibody was eluted from cellular debris together with nuclear material. The antibodies cannot simply be adsorbed on the surface of cells but are incorporated within the cells. This cytophilic antibody of small lymphocytes may well represent the immuno-memory.

In our study we were unable to obtain any information as to whether tonsils in vivo are able to be in a regional position or not, but taking into account the results of recent investigations (Oettgen et al., 1966) they do appear to be suitable for antigen reception.

RÉSUMÉ

On a examiné le rôle immunologique de l'amygdale humaine avec la méthode de la culture des tissues. Des micro-fragment de l'amygdale ont été coupés après la tonsillectomie des enfant qui auparavant ont reçu vaccinations antidiphtériques. Ces fragments ont été cultivés en milieu Parker 199. Dans ce milieu on mis de mitogen plantaire (phytohaemagglutinin). Comme antigène spécifique on employé de toxoide diphtérique. Après une incubation de 3-5 jours on pu demontrer à l'aide du test d'hémagglutination la présence des antikorps diphtériques dans la culture stimulée avec le toxoid.

ZUSAMMENFASSUNG

Die immunologische Funktion der menschlichen Tonsille wurde mit der Methode der Gewebs-Kultur studiert. Gegen Diphterie geimpften Kinder wurden tonsillektomiert: aus diesen Mandeln wurden Micro-Fragmente entfernt und in Parker 199 Nahrboden gezüchtet. Zu den Nahrboden hat man Pflanzen-Mitogen (Phytohaemagglutinin) gegeben. Als spezifischer Antigen wurde Diphtherie-Toxoid verwendet. Nach 3-5-tägige Incubation konnte man in der mit Toxoid stimulierten Züchtung die Diphtherie Antikörper mit Hilfe der Haemagglutination nachweisen.

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DISCUSSION

J G Hall. I thank the Surjáns for very interesting paper the lesson of which in my opinion must be that we become more careful when removing the tonsils.

The opponents to tonsillectomy are nowadays accumulating evidence that these peculiar organs take part in the immunological function of the lymphoid system. This was shown by Hefter and Ganz, who found esterase activity in tonsils even in adults abov 40 by Oettgen and co-workers concerning the permanent antigen supply of the tonsils, by my namesake (also concerning initials), Hall, J G with his messenger lymphocytes propagating the immune response through the body and now convincingly by the Surjans.

In Norway w are trying to investigate clinically these rather disturbing and revolutionary theories. In all cases of tonsillitis when the old question of tonsils in or out comes up, we perform a series of tests, in order to shed some light upon the urgent question: Are these tonsils infectious permanently or are they playing their role in the immunological barrier of the pharynx?

We perform bacteriological, serological, and immunological tests.

The bacteriological ones, taken from the nose and pharynx, together with the X-rays, give us a clue t whether sinusitis is present. The treatment of this sinusitis often stops the recurrent infections in the paryax, and renders tonsillectomy unnecessary W have thorough blood-examination performed in every case, and the antistreptolysin-titre is measured.

Concerning the immunological prob.em we always perform an electrophoresis of the serum and quantitative investigation of the immunoglobulins.

Besides the findings of cases of sinusitis as cause of the inflammation, we often discover cases with high AST and hypogammaglobulinaemia. When w treat this hypogammaglobulinaemia, the tonsillitis stops. In these cases there is definitely general decrease of the immuno-response.

Of course all these investigations take time, but we feel it is f importance to evaluate what at present is possible concerning the immunological status of the tonsils.

I would like very much to hear from the Surjáns some comments about the present attitude concerning tonsillectomy in Hungary

I Friedmann. What is the normal baseline adopted in the study?

L. Surján (Reply) to M *Hall*. The results of our investigations do not give any help yet about the indications of tonsillectomy W are more conservative than previously in cases of 4-6 years old children.

T Mr *Friedmann* The normal and pathological conditions are inseparable not only in cases of tonsils but also in all lymphoid organs.

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HEARING AND COMMUNICATION IN BIRDS

Species Agapornus Roseicolis

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From the E.N.T. Clinic University of Prague Prague Czechoslovakia

Allow me, please, to present to you very brief ly some results of our investigation of the communication process of two pairs of species Agapornus roseicolis in order to draw some conclusions on hearing in these birds as well as on hearing in general.

The birds studied belong to the family of parrots but, in contrast to those studied by Mr Bauk, they do not imitate human speech-sounds at all but communicate mutually with one another in their own, let us say "lan guage" which consists of very high, seemingly unmelodious and unpleasant cries in the fre quency range of between 2 000 and 8 000 cps. The distribution of sounds along the frequency le shows (Fig. 1) that there is no normal distribution but that some frequencies occur very often whereas others only exceptionally. This corresponds to the fact that the pitch of these cries is not randomized but organized into certain melodic patterns with different communicative meaning. The melodic patterns of these cries cannot be perceived by human ear because they are too high too short and the changes of the pitch are too quick. When listening to them directly the human ear inte grates all components to one mean very high pitch.

We can reveal easily the real patterns of the cries by slowing them down by means of a tape-recorder with different speeds and re producing them 4 octaves lower. Sometimes it is necessary to re-record the cries to attain the slow-down required. By this method ini tiated by the Hungarian ornithologist Peter Szoke we can transpose the birds cries or songs into the time and frequency range of human musical hearing. We see then that there are no simple bursts of high tones, but melodic patterns.

We then judged the pitch of melody pat terns subjectively by ear transcribed the melodies into notes and verified these subjective judgments by objective registration of the fundamental frequency by means of a special melograph and partially by sonagraphic analysis.

When correlating the melody patterns with the behaviour of birds and studying the re sponses of birds to the cries previously registered or synthetically formed we were able to ascertain that there is a system of communica tion, the basis of which is the movement of the fundamental frequency i.e. the pitch pat terns. Further we succeeded in recognizing the meaning of several patterns. Allow me please to demonstrate some samples of the melodic patterns in Agapornus roseicolis:

First example

The cry I call a "sthenic cry" expresses the feeling of force. It occurs before fighting or things like that (Fig. 2). It is a very common and frequent pattern consisting usually of three components the first two being low and the third one very high. The statistics of 40 ex amples of this cry show that the frequency of particular components is very exactly defined,

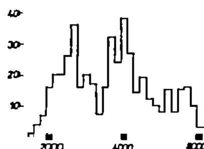


Fig 1 Distribution of sounds along the frequency scale.

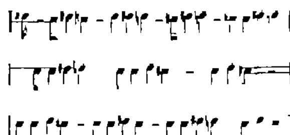


Fig 3 Sounds expressing pleasant feeling.

the standard deviation being 0 99 1.2 and 1 4 semitone. This small spread of frequency indicates a very high differentiation in production as well as selectivity in the auditory perception of the pitch.

Second example

Sounds connected with good food, expressing a pleasant feeling (Fig. 3)

Third example

Expression of displeasure and protest when their food had been taken away (Fig. 4)

Fourth example

Longing call of a she-bird when her partner left the cage and she was forced to stay inside The sonagram shows that here the particular components are separated very distinctly and that the *fundamental frequency follows the* harmonic tones, i.e. that the intervals are precise enough to form a spread chord (Fig. 5).

We can thus say that the studied birds are able to produce and hear high tones with a great precision of the absolute as well as relative pitch. They are able to produce the very quick sequences of tones of very short duration, as short as 5 msec and less. All these facts prove a very good ability of analysing the pitch of the tones. On the other hand the labyrinth of these birds contains only a relatively very short lagena with a very primitive organ of Corti. Under these conditions the place principle is inadequate as an explanation of such a perfect analysis. Consequently the direct discrimination of pitch in the central nervous system must be assumed. This has been confirmed also by the anatomical and physiological findings of Engström as well as by those of Schwarzkopff.

Communication in man differs namely in the



Fig 2. A sthenic cry

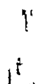


Fig 4 Expression of dis

1

1

3

2

3

Fig. 2. Muqueuse olfactive. TPNH Diaphorase positive dans l'épithélium et les glandes de Bowman (obj. 10 × 160)

Fig. 3. Muqueuse olfactive. Dehydrogenase du Glucose-6-phosphate très positive dans l'épithélium et les glandes de Bowman (obj. 10 × 160)



Fig 4 Muqueuse olfactive Dehydrogenase du lactate très positive dans l' pithelium, les glandes de Bowman et les vaisseaux sanguins (obj. 4 1 64 et obj 25 1 400)



Fig. 5. Muqueuse olfactive. α naphtyl estérase positive dans l'épithélium et les glandes de Bowman (obj. 10 × 160).

suivre nos recherches? Pour répondre à cela il est bon de jeter un coup d'œil de rappel sur le tableau des déhydrogenases intervenant dans la dégradation du glucose et voir à quel niveau se situent nos recherches précédentes (Tableau II). Notre but est en fait d'étudier à l'aide des activités enzymatiques les différents stades de cette dégradation.

Au stade actuel de nos connaissances la transformation du glucose-6-phosphate en 3 phosphate-glycéraldéhyde ne peut pas encore être étudiée directement mais il est possible de suivre cette dégradation par le biais du Shunt des Pentoses. La recherche de la déhydrogenase du glucose-6-phosphate (que nous avons déjà faite) nous renseigne sur l'activité de ce Shunt. Notons à ce propos que ce Shunt peut nous renseigner également (de loin) sur le métabolisme de l'acide nucléique.

Le stade suivant du métabolisme peut être étudié par le biais de la transformation possible du Pyruvate en lactate, transformation que nous pourrons suivre par la déhydrogenase du lactate (que nous avons également déjà étudiée). Nous comptons à ce propos compléter cette étude des lactates par la recherche des isoenzymes F (LDH 1) et des isoenzymes S (LDH 4) afin de différencier les activités mitochondriales (aérobies) des activités non mitochondriales (anaérobies).

Nous arrivons ainsi au cycle de Krebs que nous pouvons étudier en différents points. Pour ce qui concerne notre travail, nous avons déjà étudié la déhydrogenase du succinate et nous comptons compléter l'étude de ce cycle par la recherche de la déhydrogenase du glutamate qui nous renseigne par sa transformation possible en α-Cétoglutarate.

Nous croyons donc que ces quelques activités pourront ainsi nous renseigner valablement sur le métabolisme du glycogène dans les cellules de la muqueuse olfactive.

Notons pour finir que la faible activité de la phosphatase acide dans la muqueuse olfactive

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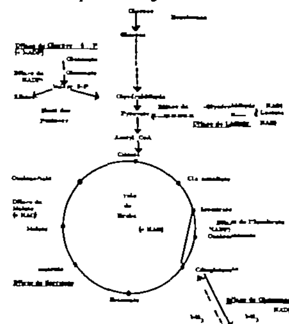
Fig 6 Muqueuse olfactive. Déhydrogénase du succinate très faiblement positive dans les glandes de Bowman (partie inférieure de la figur) contrairement à la muqueuse respiratoire (oir Fig. 7) (obj 10 1 160).

Fig 7 Muqueuse respiratoire. Déhydrogénase du succinate fortement positiv dans les glandes contrairement à la muqueuse olfactiv (voir Fig. 6) (obj. 4 1 64)

Fig. 8. Muqueuse olfactive. Phosphatase acide faiblement positive dans l'épithélium et les glandes de Bowman contrairement à la muqueuse respiratoire (voir Fig. 9) (obj. 10 : 1 — 160).

Fig. 9. Muqueuse respiratoire. Phosphatase acide fortement positive dans l'épithélium et les glandes contrairement à la muqueuse olfactive (voir Fig. 8) (obj. 10 : 1 — 160).

Tableau II *Diaphorases intervenant dans la dégradation du glucose Nous avons étudié ou nous étudierons pour la muqueuse olfactive du sujet vivant celles qui sont soulignées dans le tableau*



contrairement à son activité dans la muqueuse respiratoire peut aisément se comprendre quand on se rappelle que la phosphatase acide révèle l activité des lysosomes comme nous l avons mentionné précédemment. Ces lysosomes ont un but destructeur autodestruction des cellules malades, hétérodestruction des agents provocateurs. On peut comprendre que ces lysosomes soient bien plus actifs dans une muqueuse respiratoire beaucoup plus sujette à l'agression d'agents traumatisants extérieurs que la muqueuse olfactive, mieux protégée.

Nous remercions les Professeurs Gerebtzoff, Kluyskens et Roels qui nous aident de façon continue dans cette étude.

SUMMARY

This paper deals with the present state of the author's histochemical studies f the huma olfactory mucosa. After having described his techniques, the author gives his results obtained in material taken from human cadavers. A personal method of obtaining biopsies from the olfactory mucosa in the living is then presented and the first conclusions are discussed. Until now his method has enabled him to study the olfactory mucosa in twenty persons, by various fixation and coloring techniques and to start an investigation of the enzymes by means of frozen sections.

ZUSAMMENFASSUNG

Der Autor erläutert den gegenwärtigen Stand seiner histochemischen Studien über die Riechschleimhaut beim Menschen. Nach Beschreibung seiner Technik der Gewebeentnahme und Fixation erläutert er die Resultat die er an Leichenmaterial gewonnen hat. Sodann zieht er die ersten Schlussfolgerungen us seinen Studien am Lebenden. Seine personliche Technik der Gewebeentnahme wird geschildert. Mittels dieser Technik konnten bis heute zwanzig Lebende untersucht werden. Die entnommene Riechschleimhaut wurde mit verschiedenen Fixierungs- und Färbe mitteln behandelt. Gleichzeitig wurde anhand von Ge frierschnitten eine Untersuchung der Enzyme der Riechschleimhaut vorgenommen.

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DISCUSSION

R. Grimaud. Il convient d'insister sur les difficultés histologiques et histochimiques de la muqueuse olfac tive L'importance du rôle des enzymes trouvées par M. Willemot et provenant des glandes de Bowman est évidente et ce sont certainement les modifications qu ils subissent qui influencent le stimulus du nerf olfactif Le milieu humoral certainement une in fluence sur l'institution et l'activité des ces enzymes.

J Willemot (Reply) to M *Grimaud.* Comme M. Grimaud, je 'ai pas encore aperçu la classique tache jaune, ni sur le cadavre (frais) ni sur le vivant. Il est évident que la prochaine étape de ce travail sera de faire l'étude des enzymes de la muqueuse olfactive des sujets atteints de troubles olfactifs et de comparer cette étude à celle (que nous devrons encor compléter) faite aujourd'hui.

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DIE BEDEUTUNG VERGLEICHENDER UNTERSUCHUNGEN AM AKUSTISCHEN SYSTEM

Ch. Beck

Aus der Universitäts-Hals-Nasen-Ohrenklinik, Freiburg i. B. BRD

Abstract Zahlreiche Methoden zur Untersuchung der Physiologie und Pathophysiologie des akustischen System sind bekannt. Selten wurden aber mehrere gleichzeitig zur Aufklärung einer Fragestellung angewendet. An Hand eigener Experimente wird die Bedeutung einer vergleichenden Untersuchung am akustischen System aufgezeigt. Mit ihr sind umfassendere Aussagen z. B. über das unterschiedliche Verhalten peripherer und zentraler Abschnitte bei Intoxikation möglich als mit einer Methode allein.

Zur Erfassung der funktionellen Vorgänge an den Strukturen des akustischen Systems stehen uns heute zahlreiche Methoden zur Verfügung. Ihre Möglichkeiten und Resultate sind in grundlegenden Zusammenfassungen dargestellt (z. B. Vosteen, 1961 Rauch, 1964 Beck, 1956 Spoendlin, 1966) Jede einzelne Technik gibt Hinweise auf den metabolischen Ablauf und dessen eventuelle Störungen. Trotz dieser zahlreichen Methoden, angewandt in einer Vielzahl experimenteller Untersuchungen in den letzten Jahrzehnten, sind aber nur wenige Fakten so weitgehend gesichert daß sie eine klinische Bedeutung erlangen. Dies rührt u. E. vor allem daher daß eine Methode allein nicht genügend Aussagekraft über den Metabolismus bzw. über die biologischen Vorgängen insgesamt besitzt.

Selten war es bisher möglich, eine bestimmte Fragestellung mit verschiedenen Untersuchungstechniken gleichzeitig zu beantworten. Dies schien uns dringend erforderlich, da eine Kombination mehrerer Methoden einen besseren Einblick in die Funktionsvorgänge ermöglichen müßte als dies eine Methode allein gestattet. Wir haben deshalb seit Jahren an unserer Klinik in einem Team versucht, die uns zur Verfügung stehenden Methoden zur Untersuchung des akustischen Systems zu korrelieren. Dabei wurde nach akustischer Belastung und Einwirkung von Pharmaka die Mikropräparation der Cochlea, die Darstellung der Kerne der Hörbahnen die immunoelektrophoretische Untersuchung der Innenohrlymphe und die Registrierung akustischer Biopotentiale am gleichen Objekt angewandt. Begonnen wurde 1953 mit *morphologischen Untersuchungen* des Cortischen Organs bzw. des Ductus cochlearis und später der Kerne der Hörbahn. Ihnen schlossen sich elektrophysiologische Methoden an. Beide wurden ergänzt durch die Untersuchung der Eiweißkomponenten in Peri- und Endolymphe.

Als Ergebnis der ersten Untersuchungen konnten wir unter anderem bestätigen daß eine einmal zerstörte Sinnes- oder Ganglienzelle nicht mehr ersetzt werden kann (Abb. 1) ein Befund, den bereits Hoessli (1913) erheben konnte und der in neuester Zeit von Ruben (1969) mit Hilfe moderner zytochemischer Methoden erhärtet wurde. Weiter ergab sich, daß mindestens im Bereich der Sinneszellen des Cortischen Organs als Folge eines Stimulus oder einer Intoxikation, *Zellstadien vorhanden sein müssen, die zwar morphologisch eindeutige Zeichen einer schweren Schädigung zeigen,* aber nach Absetzen der Noxe reversibel sind. Entsprechend unseren morphologischen und elektrophysiologischen Untersu

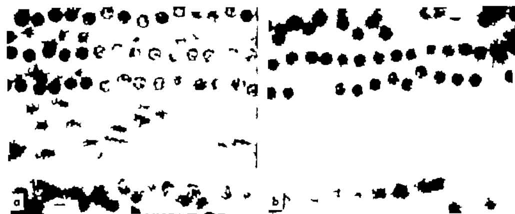


Abb 1) Normales Cortisches Organ, drei Reihen der äußeren Haarzellen gut sichtbar b) Kernlücken in der ersten Reihe der äußeren Haarzellen nach Beschallung (Feulgen 360-fach).

chungen (Beck & Michler 1960) ist dies bei den Schwellkernen der Fall (Abb 2) Diese Vorstellungen entsprechen Ergebnissen der allgemeinen Pathologie nach denen reversible Übergangsstadien zwischen Schädigung und Zelluntergang möglich sind (Ries, 1937) Sie werden untermauert durch unsere statistische Auswertung der Schädigungsrate nach Sinustonbelastung beim Meerschweinchen (Beck, 1956) (Abb 3) Während sich nach Beschallung mit 3 000 Hz, 120 dB eine Stunde sofort nach Versuchsende zahlreiche veränderte Zellen finden, sehen wir fünf Tage nach Beschallungsende nur vereinzelte Zellausfälle im Zentrum des Frequenzansprechgebietes.

Aufbauend auf diesen grundlegenden Experimenten ergab sich als Ergebnis unserer Teamarbeit (Stange et al., 1966) kurz zusammengefaßt Folgendes:

1 Durch Schallbelastung und durch ototoxische Pharmaka treten unterschiedliche Schädigungen auf die zwar nicht morphologisch, aber elektrophysiologisch im Verlauf der Adaptationsfächer sowie immunoelektrophoretisch in einer neuen Präzipitationslinie im Bereich der Alphaglobuline in Peri- und Endolymphe zu erkennen sind.

2. Die Sinneszellen des Cortischen Organs sind im Stadium der Schwellkerne nicht mehr funktionstüchtig, aber auch morphologisch unverändert erscheinende Sinneszellen können bereits eine Funktionsminderung aufweisen. So führen ototoxische Pharmaka früher zu funktionellen als zu morphologischen Beeinträchtigungen der Haarzellen, ein Befund, der sich durch eine Funktionseinschränkung der Summenaktionspotentiale ohne sichtbare morphologische Veränderungen der Sinneszellen dokumentiert. Daraus ergibt sich, daß die Registrierung akustischer Biopotentiale eine wesentliche empfindlichere Methode ist als morphologische Untersuchungstechniken.

Weiter ließ diese Teamarbeit mit Anwendung mehrerer Methoden erkennen, daß durch Ozothin, dem wasserlöslichen Oxydationsprodukt des Oleum therebinthinae, eine wahrscheinlich generelle Stabilisierung der Strukturen des akustischen Systems gegenüber Noxen



Abb 2 Rückbildung der Sch ellkerne) Kern direkt nach Beschallung. b) Z ei Stunden nach Ende der Beschallung. Geordnete Chromatinstrukturen bei Verkleinerung des Volumens

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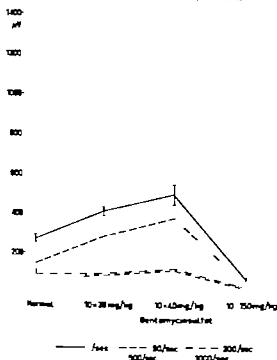
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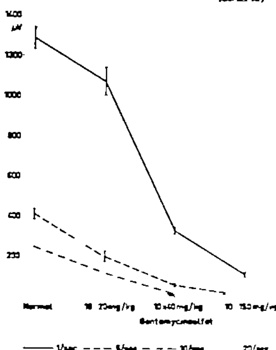


Abb. 4. Verhalten der Summenaktionspotentiale (*SAP*) des N. acusticus (*links*) und der langsamen Rindenpotentiale (*rechts*) nach Gentamycinsulfat-Intoxikation. Die SAP steigen zunächst an und fallen erst bei höheren Gentamycindosen ab, während die langsamen Rindenpotentiale sofort absinken.

Das bisher Gesagte deutet die Notwendigkeit und damit die Bedeutung vergleichender Untersuchungen des akustischen Systems an, denn Sinn und Aufgabe unserer experimentellen Untersuchungen muß letzlich sein, entweder Schäden am System zu vermeiden oder latente metabolische Beeinträchtigungen wieder zu normalisieren. Basierend auf experimentellen Ergebnissen oder auf Erkenntnissen prinzipieller metabolischer Vorgänge werden deshalb schon lange Therapieversuche bei Innenohrstörungen durchgeführt. So sei auf die Prostigmintherapie bei Ohrensausen und Innenohrschwerhörigkeit (Davis & Rommel, 1939) auf die Behandlung des Morbus Menière mit sieben-prozentiger Natriumbicarbonatlösung (Hasegawa, 1955) oder auf die Gaben von Vasodilatatoren (Bader & Beckmann, 1968 Spoendlin, 1966) hingewiesen.

Was kann uns die vergleichende Untersuchung des akustischen Systems für die Klinik in Zukunft bringen. Auf ihre Bedeutung weisen die Untersuchungsergebnisse von Suga & Snow (1969) hin, die im Experiment zeigten, daß die Gabe von Nikotinsäure bei Innenohrstörung keinerlei Effekt hat. Sie konnten die von Muscholl (1967) getroffene Feststellung bestätigen. Wir sollten also in Zukunft auf die Verwendung dieses Medikamentes verzichten. Weiter weisen solche Versuche auf die unspezifische Stabilisierungswirkung des Ozothins an der Cochlea hin, wie ich bereits erwähnte. Aufgrund der Ergebnisse unserer experimentellen Untersuchungen, aufgrund der Resultate anderer Autoren (Hasegawa, 1955 Abiko, 1968 Suga & Snow 1969) sowie unserer Überlegung über den Metabolismus der Cochlea, behandeln wir seit 1966 Patienten mit akutem

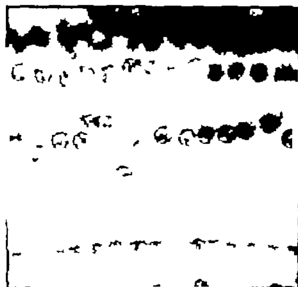


Abb 5 Unordnung der Kerne der ersten Reihe der äußeren Haarzellen als Ausdruck der Überbelastung. Gallocyanin-Chromalaun 360-fach.

Hörsturz an unserer Klinik nach einem ausgewogenen Schema (Stange, 1967) mit gutem Erfolg. Sicher beinhaltet diese Therapie keine Erfolgsgarantie *sie beschränkt sich aber auf* wenige Medikamente, von denen wir wissen oder zu wissen glauben, daß sie einen günstigen Einfluß auf die Strukturen bzw des Metabolismus des akustischen Systems haben. Dabei ist zu bedenken daß die erwähnte Reversibilität geschädigter Sinneszellen die eine Möglichkeit ist, die andere ist der Zelltod. Deshalb muß damit gerechnet werden, daß nach Absetzen der Noxe die Schädigung ihr endgültiges Ausmaß noch nicht erreicht hat. Unsere Therapie hat sich danach zu richten, d. h. sie muß über längere Zeit durchgeführt werden, eine Überlegung, die besonders die Dauer unserer Behandlung bei der akuten Hörminderung mitbestimmt hat.

Wir stehen heute am Anfang einer gezielten Therapie des akustischen Systems und haben noch viel zu erarbeiten und zu erforschen. Es sei an die zentralen Bahnen erinnert, deren Problematik ich angedeutet habe, da wir hier über äußerst spärliche Kenntnisse besitzen. Zur weiteren Aufklärung scheint uns die Korrelationsmöglichkeit vieler Untersuchungsmethoden von Bedeutung, denn sie kann uns wesentlich mehr Informationen vermitteln, als eine Methode allein. Sie wird uns vor Fehldeutungen bewahren und uns gleichzeitig ermöglichen, eine gezielte und dadurch effektive Innenohrtherapie durchzuführen.

Tabelle I *Therapieschema bei akutem Hörsturz*

Akute Hörstürze Therapie Medikation	Dosis
7 % $NaHCO_3$-Infusion	jeden 2. Tag 250 ml
Stellatum-Blokade	jeden 2. Tag 10 ml Xylocain
Complamin	täglich 3 2 Tbl. (evtl. i.)
Ozothin	täglich 3 1 Supp. (evtl. i.m.)
Neurobion	täglich 3 1 Drag.

SUMMARY

A great number of methods for the investigation of the physiology and pathophysiology of the acoustic system are known but they are seldom applied together in order to solve one single problem. I our own experiments we demonstrate the importance of correlated investigations into the function of the acoustic system. They show more comprehensive observations e.g. f the various reactions of inner ear cells and central regions to an intoxication than one single method can show

RÉSUMÉ

De nombreuses méthodes pour examiner le fonction de système acoustique sont bien connues, mais elles ne sont que rarement appliquées ensembles pour resoudre un seul problème. Dans nos observations nous démontrons l'importance des recherches comparatives du système acoustique. Pour une intoxication par example, elles nous donnent des résultats plus exactes des différentes réactions cellulaires périphériques et centrales que pourrait en donner une seule méthode.

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DISCUSSION

F Zollner Ich darf, da die Ausführungen Kollegen Beck's die in sehr knapper Form eine Fülle von Ergebnissen brachten, nur einige ergänzende Hinweise erlauben. Bei allen Tierversuchen mit Schall einwirkung auf das Innenohr wurde ein Mikrophon operativ an den Gehörgang befestigt und der Schalldruck am Trommelfell gemessen. Bei Beschallung mit Lautsprechern gegenüber dem frei beweglichen Kopf des Tieres können grosse Abweichungen von der angenommenen Dosis entstehen. Veranderungen der elektrophysiologischen Reaktion sind früher zu registrieren als morphologische und ergaben daher für die Zusammenarbeit eine ganz besonders empfindliche und zuverlässige Kontrolle. Man soll daher künftig nicht versäumen bei den für die Klinik so wichtigen Versuchen über akustische und toxische Schaden am Innenohr und deren Behandlung diese exakten, kombinierten Methoden anzuwenden.

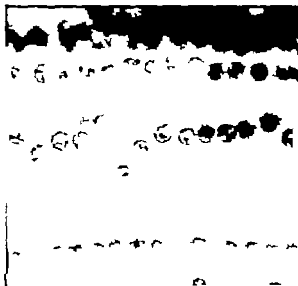


Abb 3 Unordnung der Kerne der ersten Reihe der äußeren Haarzellen als Ausdruck der Überbelastung. Gallocyanin-Chromalaun 360-fach.

Hörsturz an unserer Klinik nach einem ausgewogenen Schema (Stange, 1967) mit gutem Erfolg. Sicher beinhaltet diese Therapie keine Erfolgsgarantie, sie beschränkt sich aber auf wenige Medikamente, von denen wir wissen oder zu wissen glauben, daß sie einen günstigen Einfluß auf die Strukturen bzw des Metabolismus des akustischen Systems haben Dabei ist zu bedenken, daß die erwähnte Reversibilität geschädigter Sinneszellen die eine Möglichkeit ist, die andere ist der Zelltod Deshalb muß damit gerechnet werden, daß nach Absetzen der Noxe die Schädigung ihr endgültiges Ausmaß noch nicht erreicht hat. Unsere Therapie hat sich danach zu richten, d. h. sie muß über längere Zeit durchgeführt werden, eine Überlegung, die besonders die Dauer unserer Behandlung bei der akuten Hörminderung mitbestimmt hat.

Wir stehen heute am Anfang einer gezielten Therapie des akustischen Systems und haben noch viel zu erarbeiten und zu erforschen. Es sei an die zentralen Bahnen erinnert, deren Problematik ich angedeutet habe, da wir hier über äußerst spärliche Kenntnisse besitzen. Zur weiteren Aufklärung scheint uns die Korrelationsmöglichkeit vieler Untersuchungsmethoden von Bedeutung, denn sie kann uns wesentlich mehr Informationen vermitteln, als eine Methode allein. Sie wird uns vor Fehldeutungen bewahren und uns gleichzeitig ermöglichen, eine gezielte und dadurch effektive Innenohrtherapie durchzuführen.

Tabelle I *Therapieschema bei akutem Hörsturz*

Akute Hörstörung Therapie Medikation	Dosis
7% $NaHCO_3$-Infusion	jeden 2. Tag 250 ml
Stellatum-Blokade	jeden 2. Tag 10 ml Xylocain
Complamin	täglich 3 2 Tbl (evtl. i.v.)
Ozothin®	täglich 3 1 Supp. (evtl. i.m.)
Neurobion®	täglich 3 1 Drag

SUMMARY

A great number of methods for the investigation of the physiology and pathophysiology of the acoustic system are known but they are seldom applied together in order to solve one single problem. In our own experiments we demonstrate the importance of correlated investigations into the function of the acoustic system. They show more comprehensive observations e.g. of the various reactions of inner ear cells and central regions to an intoxication than one single method can show

RÉSUMÉ

De nombreuses méthodes pour examiner le fonction de système acoustique sont bien connues, mais elles ne sont que rarement appliquées ensembles pour résoudre un seul problème. Dans nos observations nous démontrons l'importance des recherches comparatives du système acoustique. Pour une intoxication par example, elles nous donnent des résultats plus exactes des différentes réactions cellulaires périphériques et centrales que pourrait en donner une seule méthode

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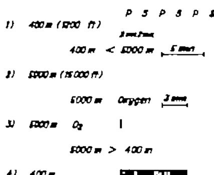


Fig 1 Sequence of tracings during experiment.

to about 85 mm. After a pause of 5 min, threshold tracings are made under these hypoxic conditions. Thereafter oxygen is administered by a rubber tube or an oxygen mask and the subject registers further threshold tracings. The test ends with some control tracings after having regained the initial level of 400 m. The whole experiment lasts about 1 $/_2$ hours, its application is of course limited to healthy persons.

Hypoxia corresponding to a level of 5 000 m may influence the psychic behaviour of the subjects to an individual degree. Some are depressed and suffer from a precollapse state, others feel consistently well and some are even euphoric.

Different parameters are considered (Fig. 2):

(*a*) Threshold for pulsed an steady signals.

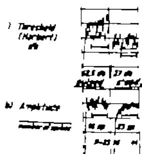


Fig 2 Parameters evaluated in the experiment (*p* pulsed; *s*, steady).

Various suggestions have been made for deriving the threshold values from Békésy Jerger tracings. In agreement with Harbert & Young (1966) we prefer to use the bottom of the curves when evaluating threshold variations.

A difference between the threshold for pulsed and for steady signals ("separation") means an impaired slow adaptation resp positive tone decay (Harbert & Young, 1966) Since it was well known that the subject needs about 30 sec to produce regular tracings (Bradford & Goetzinger 1964) the last minute of each 2 min period was selected for measuring hearing loss.

(*b*) Amplitude of steady signal vs. pulsed signal tracings. Instead of measuring each single pen trace, it is much easier to count the number of pen-swings for a given period of time. This value is inversely proportional to the amplitude (Harbert & Young, 1966 Silbiger & Elliott, 1966) Not the absolute number of pen-swings resp. the absolute value of the amplitude but the relation of the figures for steady to pulsed signals is representative for an impaired rapid adaptation.

RESULTS

1 (*a*) *Amplitude of pulsed and steady continuous frequency tracings*

During preliminary experiments we experienced that the amplitude of both steady and pulsed signal tracings increased under hypoxia. This phenomenon must be related to the mental state of the subject under influence of oxygen deficiency We do not think that it is directly related to adaptation itself.

1 (*b*) *Amplitude Ratio of steady vs pulsed signal tracings*

The reduction of amplitude in steady signal tracings indicates a loss of rapid adaptation. As the above-mentioned increase in amplitude under hypoxia logically pertains to both pulsed and steady tracings, a narrowing of the amplitude for steady signals can only be prov

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AUDITORY ADAPTATION IN HYPOXIA

G von Schulthess

From the Aeromedical Institute of the Swiss Air Force, Dübendorf Switzerland

Abstract Hypoxia corresponding to an altitude of 5 000 m may influence auditory adaptation at 4 000 cps to an individual degree. Only rapid adaptation seems to be affected by oxygen deficiency whereas no measurable changes of slow adaptation can be found.

Hypoxia does not seem to influence the *pure tone threshold* in humans unless mental functions like attention and concentration are heavily disturbed. But what happens to *auditory adaptation* under hypoxic conditions?

We know that the *eye* adapts in a biphasic way (alpha and beta adaptation, Wright, 1959), and its rapid or alpha adaptation appears to be a neural mechanism. McDonald & Adler 959) found that this type of adaptation of q eye is affected by hypoxia. In the *ear* also ere are two forms of adaptation (Hood 1950 Harbert & Young, 1964) on-effect or rapid, adaptation and slow adaptation or per-stimulatory fatigue. Harbert & Young (1964 1966) used v Békésy (1947) and Jerger's (1960) fixed frequency threshold tracings for pulsed and steady signals in order to demonstrate these adaptation mechanisms of the ear They found a correspondence of the amplitude in steady signal tracings with rapid adaptation and of the threshold difference between steady and pulsed signal tracings with slow adaptation resp. tone-decay Both mechanisms take place in the brain-stem and most likely in the efferent system (Blegvad, 1968 Hahn & Scarzella, 1960).

We have attempted to demonstrate the influence of hypoxia on auditory adaptation by testing with an automatic Békésy Jerger audiometer

The experiments are made in a pressure cabin. The test-subject with ear-phones and controlbutton is placed inside the cabin, whereas the experimentor controls a Kamplex AB Békésy Type Audiometer outside. Communication between the two persons is possible through an observation window and by telephone

A 4 000 cps signal alternately pulsed and steady in character is applied to one ear of the subject the other ear is covered by an ear phone and is not masked. On- and off time of the pulsed signal are kept at 200 msec. The intensity variations are of 2.5 dB/sec and the registration sheet is displaced at a velocity of 1 cm/min. At the beginning of each period of pulsed and steady signal tracings the signal is given at the maximal output intensity of the instrument. Originally it was planned to include a 1 000 cps signal in the study but for reasons of the duration of each test, this part of the project had to be abandoned.

Fig. 1 shows a diagram of the course of the experiment. During one or more preliminary runs, the subject has the opportunity of getting acquainted with the audiometric test. Then the cabin is closed and two pairs of tracings, pulsed and steady are registered at a normal pressure of 400 m above sea-level. Now the cabin is depressurized to a simulated altitude of 5 000 m (i.e. 15 000 ft) At this level the blood oxygen pressure is supposed to be lowered from the normal value of 160

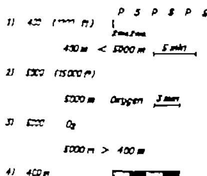


Fig. 1. Sequence of tracings during experiment.

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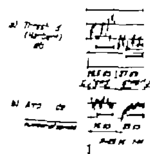


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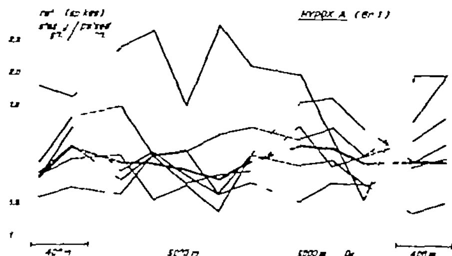


Fig 3 Group 1 (males ?0 years): Rapid adaptation under experimental conditions (5 ordinary cases with mean value, 1 exceptional case).

relating its figures to those of pulsed signals. In terms of the number of pen-reversals this factor of steady vs. pulsed should increase. In a group of young students this phenomenon was not constantly present (Fig. 3) whereas it was slightly indicated in a group of older subjects (Fig. 4) two of whom had a high tone loss. In one of the students we had the opportunity to reproduce the tracings (Fig. 5). We want to stress that the effect of hypoxia varies individually and even in the individual subject, the effect on adaptation is not constant.

Normal adaptation was not always regained after return to oxygen supply or normal atmospheric conditions.

2. *Threshold shift of steady compaired with pulsed signal tracings*

This relationship allows us to demonstrate the absence or presence of slow adaptation (Harbert & Young, 1964). We find an elevation of the threshold under hypoxia both for steady and for pulsed signals. But a separation of the threshold values under hypoxia was not present in either group of subjects (Fig. 6). It is possible that the increase and decrease in atmospheric pressure which is run through in a very short period of time under the test conditions can influence sound condition and therefore interfere with the threshold measurements.

COMMENT

The fixed frequency tracings for pulsed and steady signals at 4 000 cps under hypoxic conditions demonstrate remarkable variations. Certain cases which we cannot specify at the moment, show an impaired rapid adaptation under hypoxie. A statistical evaluation of this phenomenon was not possible as the number

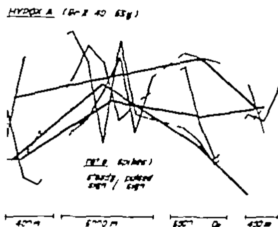


Fig 4 Group (males 40–65 years): Rapid adaptation under experimental conditions; 3 subjects and mean values for each singular experimental period (400 m – 5 000 m – 5 000 m + O_2 – 400 m), continuous line.

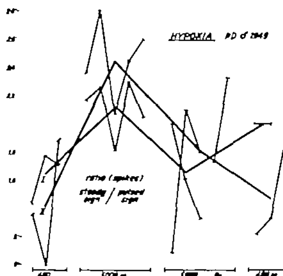


Fig 5 P.D male 20 years: Rapid adaptation during experiment, Reproducibility (I&II) Mean values for each experimental period.

of observations is too small. The individual variations of the reactions to oxygen deficiency the oscillations in the reactions of the subject and the actual mental state of each subject under test may explain this lack of consistency Blegvad s (1968) experiments whith noise exposure of the contralateral ear and Hahn & Scarzella s (1960) pharmacological tests pertain to the same problem as ours, i.e. adaptation phenomena under specified conditions. Blegvad (1968) also found variations in individual behaviour After noise exposure the return to pre-experimental values of amplitude was often delayed or even not demonstrable within the test conditions. Analogously after resumption of oxygen supply we did not always find an immediate recovery of rapid adaptation.

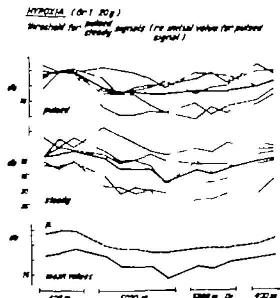


Fig 6. Group 1 (males 20 years): Threshold values for pulsed and steady signals.

An impaired slow adaptation, which should have been demonstrated by separation of threshold tracings for steady in relation to pulsed signals, was not present under hypoxia. Nor was a delayed recovery of the threshold found after exposure to maximal intensities of the signal. More information on the adaptive behaviour under hypoxia of patients with cochlear or retrocochlear hearing loss should be gained.

ACKNOWLEDGMENTS

The pressure cabin was kindly placed at our disposal by the Aeromedical Institute of the Swiss Air Force and the Physiological Institute of the University of Zurich (Switzerland) We are very grateful to Dr E. Hardmeyer and Prof. Dr O Wyss for their kind support. The Kamplex Békésy Type Audiometer has been kindly lent to us by Prof. K. Graf, E.N.T clinic Lucerne. The assistance of Mr G Guinard and Mr H. Jenni was invaluable.

RÉSUMÉ

L'influence de l'hypoxie sur l'adaptation auditive est examinée dans une cabine à décompression Les tracés de l' udiometre automatique de Békésy à fréquence fixe manifestent une altération de l'adaptation type rapide dans des conditions hypoxémiques tandis que le seuil des tracés reste inchangé.

ZUSAMMENFASSUNG

Der Einfluss einer Hypoxie, welche den atmosphärischen Verhaltnissen auf ca. 5 000 m.ü.M entspricht, uf die Gehorsadaptation wird untersucht. Mit der Békésy-Jerger'schen automatischen Audiometrie wird eine gestorte Kurzzeitadaptation nach-

gewiesen, während eine Hörschwellenverschiebung im Sinne der Langzeitadaptation nicht gefunden wird.

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DISCUSSION

M Portmann. Parmi les différents facteurs qui interviennent sur l'audition, il y a dans cette expérience non seulement l'hypoxie mais aussi la modification de la pression de l'atmosphère. M. Schulthess a-t-il fait la mesure de O² du sang? A-t-il fait d'autres études en atmosphère normale avec un masque respiratoire pour changer la pression de l'oxygène respiré?

P Menzio It is well known that hypoxia produces remarkable electrophysiological modifications both in the cochlea and in the central auditory pathways, but the most meaningful take place in the cochlear potentials. The rapid disappearance of the neural components N_1 and N_2 suggest that the first cochlear neuron is extremely sensitive to oxygen deficiency and has therefore a very strong metabolism, more than that of the other peripheral nerves. During hypoxia and recovery a different behaviour has been noticed, varying according to the stimulus intensity This shows there are sets of fibers with varying oxygen requirement and agrees with the observations of Davis, Tasaki and others on the existence of two sets of fibers inside the Corti ganglion. Most of them have a low threshold and an increase in discharge frequency small related to others, fewer have a higher threshold and a rapid increase in discharge frequency (small dynamic range"). Do you think such observations are meaningful in order to explain the results?

G von Schulthess (Reply) to Mr *Portmann.* Chemical blood tests involving arterial punctures would have been refused by the subjects, but we believe that maintenance in a highly hypoxic ambience for more than 1 hour reduces the likelihood that factors other than hypoxia influence the test result. Experiments with hypooxygenation by means of a mask had to be abandoned as impracticable during preliminary tests.

To M *Menzio* It is quite possible that an individual sensitivity to oxygen loss in certain neurons or ganglia plays a part in the individual variations of the test results, though there should be evidence of such variations, probably in animal experiments.

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FURTHER OBSERVATIONS UPON THE NEUROLOGICAL MECHANISM OF OPTOKINETIC NYSTAGMUS

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Abstract Clinical studies of patients with lesions of the cortex and basal ganglia have provided support for the existence of two distinct mechanisms for the fast and slow components f optokinetic nystagmus with centres in the frontal and occipital cortex respectively and for cortical association pathway between the two centres.

One of the most consistent and invariable aspects of optokinetic nystagmus elicited by means of a large striped revolving drum enclosing a normal subject is the deviation of the eyes in the direction of the fast component. This finding is so constant that any departure from it can be regarded as abnormal. Typical tracings are shown in the upper part of Fig. 1

The direction of the drum is reversed at the points shown by the arrows and it will be seen that this results in an immediate reversal of the nystagmus beginning with a fast component which takes the eyes past the mid-line There after all nystagmic movements occur with the gaze deviated laterally in a direction opposite to that of the movement of the drum. The lower tracing, by contrast, is of vestibular nystagmus induced by a torsional rotational stimulus and recorded with the eyes open in darkness. The deviation of the eyes in the direction of the fast component is absent and, furthermore, the change in direction of the nystagmus begins with a change in direction of the slow component.

Hood in an earlier communication to the Collegium (1967) drew attention to this particular feature of optokinetic nystagmus and concluded that it implied that the fast component was not simply a reflex return of the eyes to the mid-line position as had been held by Kestenbaum (1948) Rademaker & Ter Braak (1948) and others but was a manifestation of high order cortical activity involving the frontal centre for voluntary gaze closely akin, in all probability to the fixation reflex. This conclusion was supported by subsequent observations upon subjects with central scotomata in whom he showed that in the absence of foveal vision, resulting presumably in the inactivation of the nervous pathways concerned with fixation, the optokinetic nystagmus took on the features, as shown in Fig. 2, of reflex vestibular nystagmus characterised by a deviation of the eyes in the direction of the slow component and a change in direction of the slow component.

Since then the results of these and similar observations have led to the formulation of a simple hypothesis of the nervous mechanism of optokinetic nystagmus which though clearly speculative and incomplete provides a serviceable basis for the integration of the relevant anatomical, physiological and clinical data.

By way of illustration let us first consider the simple situation of a subject seated in darkness inside the revolving drum. At the moment the interior of the drum is illuminated, the nystagmus characteristically takes on the form shown in Fig. 3 Initially the nystagmus begins

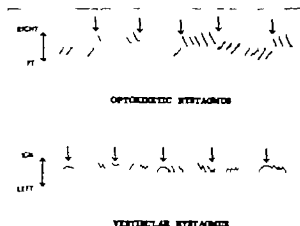


Fig 1 Recordings of optikinetic and vestibular nystagmus showing characteristic changes upon reversal of direction of nystagmus.

with a slow component which deviates the eyes in the direction of rotation of the drum. This slow component lasts some 200 milliseconds and its velocity is always considerably less than the velocity of subsequent slow components. Thereafter it is followed by a fast component which deviates the eyes in the opposite direction of gaze and it is in this direction that all subsequent nystagmic movements take place.

In Fig. 4 are shown the pathways that we suppose to be involved in these events.

Afferent impulses from both the macula and peripheral retina are conveyed by way of the visual pathways to the occipital lobe from

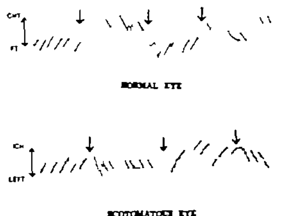


Fig 2 Recordings of optokinetic nystagmus from a patient with a unilateral central scotoma. Upper tracing from normal eye with impaired eye covered. Lower tracing from impaired eye with normal eye covered.

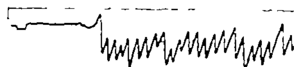


Fig 3 Recording showing initiation of optokinetic nystagmus, (normal subject) upon illumination of revolving optokinetic drum. *Note* initial slow component.

which in turn efferent impulses to the oculomotor nuclei reflexly bring about the slow movement of the eyes. The very slow velocity of this initial slow component indicates that there is a considerable difference between the actual drum velocity and that of the eyes and as a result there must occur a marked slipping of the images of the stripes across the retina. In other words the fovea can be presumed to play no active part in this initial response. At this moment in time, however impulses are conveyed to the frontal visual centre which subsequently by way of its own separate and distinct projection pathway to the ocular muscle nuclei, initiates the fast component. Thereafter *in subsequent nystagmic movements the* frontal visual centre takes on the dominant role and the eyes remain deviated in the direction of the fast component.

In addition it seems necessary to add one further pathway first proposed by Holmes (1938) which subserves an inhibitory controlling influence exerted by the frontal centre upon inappropriate and undesirable activity of the occipital lobes.

The above explanation imputes two separate and distinct pathways and mechanisms—one for the slow component and the other for the fast component together with sub-cortical association fibres connecting the frontal and occipital centres for eye movement.

Needless to say the scheme presented here is obviously incomplete and account must also be taken of cerebellar regulatory (Fuchs & Kornhuber 1970) and other influences.

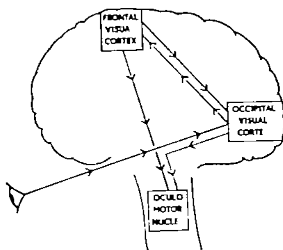


Fig 4 Schematic illustration of suggested nervous pathways subserving optokinetic nystagmus.

Before describing our clinical material we may review briefly what anatomical and experimental evidence is available to support the existence of these pathways. This may be considered under three headings:

Pathway for the fast component

A non-crossed cortico-diencephalo-mesencephalic path has been extensively studied by Brucher (1964) in cortical studies and by Bender & Shanzer (1964) in brain stem studies upon monkeys. The fibres are associated with the frontal visual cortex (Broca's Area 8) and other cerebral areas and have been traced through the globus pallidus, ventrolateral part of the thalamus, zona incerta and Fields of Forel. The left and right pathways converge in the pretectum and in the mid brain are located in the paramedian zone ventrolateral to the medial longitudinal bundle crossing the mid line at the level of the oculomotortrochlear nuclei and continuing in the paramedian zone of the pontine tegmentum. The mesencephalic and pontine portions of this pathway occupy the general regions of the excitatory or activating reticular formation.

Pathway for the slow component

The occipital eye field has long been known to be concerned with visual reflexes including those induced by following a moving object. The associated cortico-mesencephalic pathway involved was shown by Mettler (1935) to be associated only with striate cortex responsible for peripheral vision and not from the macular region. The efferent fibres lie parallel with and medial to the visual radiation and pass through the posterior end of the internal capsule being distributed to the nucleus of Darkschewitch, interstitial nucleus of the medial longitudinal bundle (Cajal) and oculomotor nuclei. Other fibres have been traced by Crosby & Henderson (1948) to the superior colliculi.

Subcortical association pathway

Association fibres between the homolateral frontal and occipital eye centres have been demonstrated by Claes (1939) and by Crosby (1962). The occipital lobe has been shown to exert a tonic depressant effect on the frontal eye centres. Electro-encephalographically moreover lesions of the occipital lobe produce confusing disturbances in the electrical activity of the frontal area (Cogan, 1956). Hood (1967) showed furthermore that subjects without macular vision were able to follow a rotating striped drum to much higher velocities than normal by virtue of the uninhibited occipital centre.

This scheme, although clearly incomplete, goes some way towards explaining some of our results already described. Thus lesions involving the integrity at any point in the pathways subserving foveal vision would be expected to modify the resultant nystagmus. The results from the cases of central scotomata are illustrative of this. Here foveal vision is nonexistent and therefore pathways to and from the frontal centre can exert no practical effect. The outcome therefore is a nystagmus which possesses the reflex characteristics of vestibular nystagmus with the eyes deviated in the direction of the slow component. Now on this basis it is to be expected that lesions involving the central pathways to and from the frontal centre will result in precisely the same disordered featu

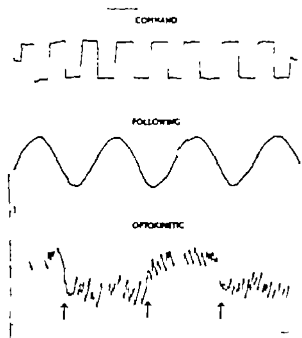


Fig 5 Command, following and optokinetic responses in a normal subject.

of optokinetic nystagmus as we have encountered in central scotomata.

Recently through the good offices of our colleagues at Queen Square we have had the opportunity of examining a variety of cases in whom there is good reason to suppose that the pathways subserving voluntary fixation, and consequently the fast component of optokinetic nystagmus, have been implicated. All cases were subjected to an electro-nystagmographic examination of which typical results for a normal subject are shown in Fig. 5 Firstly saccadic movements on command between points 30 to subject s left or right, secondly following movements and thirdly optokinetic nystagmus with the subject seated inside a large rotating drum, particular attention being paid to the events taking place on reversal of the direction of the drum.

Two groups of patients were examined. The first group consisted of some 20 patients suffering from progressive degenerative conditions with a pathology centered around the basal ganglia and anterior mid brain. The following diagnosis had been made·

Huntington's Chorea	5 cases
Progressive Supranuclear Palsy	9 cases
Arteriosclerosis involving basal ganglia	4 cases
Familial ataxias	2 cases

All these cases exhibited certain characteristic disorders of eye movements. In the majority there was some limitation of gaze· in all, optokinetic nystagmus elicited with a small Bárány drum was either absent or grossly reduced, and in all it was possible to show that there was a marked slowing of saccadic movements on command.

While therefore, the possibility cannot be excluded that the projection pathways from the occipital lobes subserving the slow component were involved by the lesion, in all cases there was clear evidence that the pathways from the frontal visual cortex subserving the fast component had been deranged.

Typical electro-nystagmographic findings in one of these cases (for clinical details see appendix) are shown in Fig. 6. As can be seen, command movements are executed extremely slowly following movements are irregular and finally the optokinetic response is grossly deranged and characterized by a deviation of the eyes in the direction of the slow component. The tracings shown in Fig. 7 are also taken from a patient in this series (for clinical details see appendix) but in whom the derangements were less marked. Nevertheless the deviation of the optokinetic response in the direction of the slow component is still present.

We may conclude therefore that in these cases the deviation of gaze in the direction of the slow component was the result of interference with the pathways subserving the fast component, thus nullifying the influence of the frontal visual centre.

Our second group consisted of 2 patients with disease of the cerebral hemispheres. One was diagnosed as Jakob Creutzfeldt disease, the other as multiple cerebral emboli.

The striking feature of these 2 cases was that on clinical examination no disorder of eye movements was apparent. Optokinetic nystag

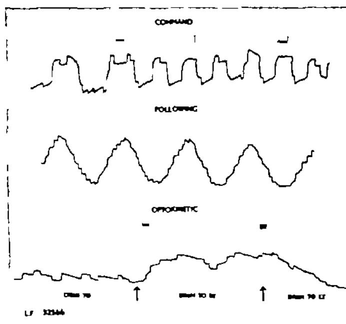


Fig. 6 Command, following and optokinetic responses in a patient with basal ganglion lesion. Case 1

mus with a small Bárány drum appeared normal as did command and following movements. *The electro-nystagmographic findings in both* cases were similar and the results for one are shown in Fig. 8 (for clinical details see appendix)

As *can be seen, command movements,* although stepped, are executed with normal velocity Following movements are normal. The optokinetic response however though brisk and with an appearance of normality is in fact *clearly abnormal since the eyes deviate not in* the direction of the fast component but in the direction of the slow Our presumption in these 2 cases is that the lesion has involved the pathways *from the frontal to the occipital cortex.*

The scheme that we have put forward is not of course new Bárány (1921) was perhaps the

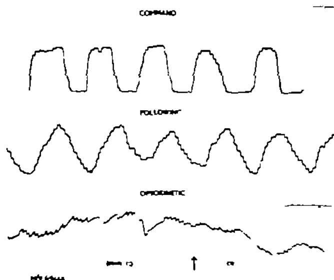


Fig. 7 Command, following and optokinetic responses in patient with basal ganglion lesion. Case 2.

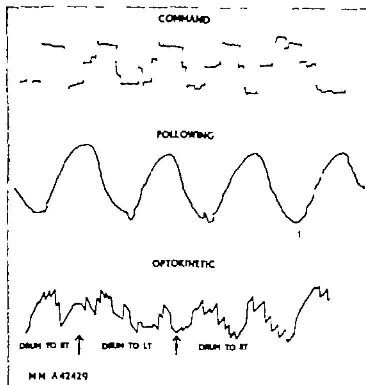


Fig 8 Command, following and optokinetic responses in a patient with cerebral lesion affecting frontal gaze centres. Case 4

first to put forward the suggestion that the slow phase was initiated from the angular gyrus and the fast from the frontal lobe. Borries (1926) also postulated two cerebral centres, one in the l lobe and the other in the frontal lobe ith intracerebral connections between them. More recently Ling & Gay (1968) have elaborated upon this theme in greater detail and their argument accords with the suggestion we have put forward.

Criticisms that have been raised in the past about the role of the frontal centres in initiating the fast phase rest upon the argument that with bilateral frontal ablation, both in man and animals, optokinetic nystagmus is unimpaired (Feldman & Bender 1969) In the light of our present findings this does not seem to present a serious objection.

In both groups of our cases the mechanism of the fast component has been implicated, yet optokinetic nystagmus still persists to a greater or lesser degree and indeed in the second group would appear to be normal on direct observation of the eyes. Nevertheless both groups exhibit an abnormal deviation of the eyes in the direction of the slow component. This we regard as being highly significant and indicative of the removal of the influence of the frontal centre. It remains to be explained why a fast component should persist in these cases. Our suggestion is that since the optokinetic nystagmus in these patients has the characteristics of reflex vestibular nystagmus, the role of the *frontal centre in initiating the fast component* has been taken over by the same mechanism responsible for the fast component in reflex vestibular nystagmus and is dependent upon the integrity of the pontine reticular nuclei. It might well be that in normal circumstances the same mechanism for the fast component is shared both for optokinetic as well as vestibular nystagmus but in the case of the former it is under the controlling influence of the frontal visual centre Beyond this little more can be said. Our results, nevertheless, incomplete as they are do suggest that the frontal visual centre exerts a powerful controlling influence upon optokinetic nystagmus. For the detection

of this influence furthermore, clinical observation of optokinetic nystagmus is not sufficient. We must in the future depend upon a critical analysis of recorded eye movements with particular reference to the precise details outlined above.

APPENDIX

Group I

Case 1 L. F (N H A52566)

A man aged 62, admitted under the care of Dr Ross Russell, had experienced difficulty with reading, being unable to find the next line down, for 2 years. His family noted slurring of speech, fatigue, imbalance on going about and difficulty with vision, with inability to focus. He had been unable to drive or see stairs and tended to stumble. For the past year he had been falling backwards.

Neurological findings

He had a mild memory loss, monotonous voice and mild bilateral facial weakness. Pupils, fundi and fields were normal. The neck was rigid, the limbs were spastic with increased reflexes

Neuro-otological findings

The patient was unable to stand with closed eyes, falling backwards and to the right, and was unsteady on walking, drifting to the right. Eye movements were as follows·

Command and following movement upwards was incomplete in the vertical plane being limited to some 45 upwards and 5 downwards. Right lateral gaze was complete. Adduction of right eye was incomplete on left lateral gaze.

Convergence: absent.

Dolls head movements. complete to left and right, up and down.

Spontaneous nystagmus: coarse 1st degree vestibular type nystagmus was present to left and right, that to left being dissociated in type, more marked in left eye with incomplete adduction of right eye

Positional nystagmus: absent.

Optokinetic nystagmus (small drum) responses to left and right feeble. Responses absent in vertical plane the eyes deviating upwards with upward and downwards with downward rotation of drum.

Caloric test, Fitzgerald and Hallpike technique· responses exaggerated, normal pattern.

Bilateral simultaneous stimulation, 20 C (1 min)· tonic deviation of eyes downwards $2^1/_2$ min no nystagmus, 45 C (1 min)· tonic deviation of eyes upwards $2\,^1/_2$ min, no nystagmus.

Electro-nystagmographic examination (large drum) optokinetic responses grossly deranged. Eyes deviated markedly in direction of slow component of nystagmus (Fig. 6)

Comments and diagnosis

Progressive supranuclear palsy with ophthalmoplegia complete for convergence and partial for gaze in the vertical plane more severe for downward than upward movement. The fast components of optokinetic and vestibular nystagmus were absent in the vertical plane. Optokinetic nystagmus to left and right was feeble By contrast, dolls head movements and the slow components of vestibular nystagmus in the vertical plane were intact. The right internuclear ophthalmoplegia would indicate extension of the lesion to involve the right medial longitudinal bundle between the 3rd and 6th nerve nuclei.

Case 2 M H (M V H 69148)

A women, aged 64 was admitted under the care of Dr Marshall with 2 years history of clumsiness and stiffness of the right arm and unsteadiness in walking. During the past 6 months she had fallen backwards several times and been unable to go out alone. She had also noted difficulty in reading.

Neurological findings

The pupils reacted sluggishly to light both directly and consensually Fundi and fields were normal Speech was slow and slurred and the

neck stiff and rigid. There was hypertonia and weakness of the right arm of extrapyramidal type with cogwheel rigidity and marked incoordination. Reflexes were increased in both legs. The right plantar response was extensor. Psychological tests revealed mild intellectual impairment.

Neuro-otological findings

The patient tended to lean backwards but was able to stand and walk stiffly on a wide base with the eyes closed. Eye movements were as follows:

Command and following: no movement up or down. Some 5° of movement only to left and right were present on occasions. This varied being sometimes almost full and better to left than right.

Convergence: absent.

Dolls head movements: complete to left and right, up and down.

Spontaneous and positional nystagmus: absent.

Optokinetic nystagmus (small drum): responses absent to left and right, up and down.

Caloric test, Fitzgerald and Hallpike technique: responses of normal and equal duration but abnormal in character consisting of tonic ocular deviations in direction of slow component with absent fast component to right and very feeble fast component to left. Bilateral simultaneous stimulation, 20° C (1 min): tonic deviation of eyes downwards 3 min, no nystagmus. 46° C (1 min): tonic deviation of eyes upwards 2 1/2 min, no nystagmus.

Electro-nystagmographic examination (large drum): optokinetic nystagmus grossly deranged. Eyes deviated markedly in direction of slow component of nystagmus (Fig. 7).

Comments and diagnosis

Progressive supranuclear palsy with complete ophthalmoplegia for convergence and movements in vertical plane and incomplete in lateral plane being more severe to right than left. The fast components of optokinetic and vestibular nystagmus were absent in the vertical plane and severely deranged to left and right, more so to right. By contrast dolls head movements and the slow components of vestibular nystagmus were preserved in all directions.

Group II

Case 3 L. J (N H A35803)

A man of 57, a professional pianist, under the care of Dr Ross Russell, presented with 4 month's history of spasmodic attacks of difficulty with speech. Nine weeks ago he had lost his speech entirely and had trouble with swallowing, chewing and coughing, sometimes regurgitating fluids through his nose. For 2 months he had been unable to co-ordinate his hands and was now unable to play the piano. He had also been unsteady on his feet. He tended to laugh and cry with little provocation.

Neurological findings

Alert and co-operative. Emotionally labile. Impaired higher mental function. Verbal I.Q. 113. Performance I.Q. 88. Variable impairment of conjugate gaze and difficulty in looking in a particular direction on command. Fundi, fields and pupils were normal. No diplopia or nystagmus. Minimal weakness of shoulders, right worse than left. Normal tone but inconstant fasciculation in both calves. Alternating movements performed clumsily. All reflexes pathologically brisk. Unsustained ankle clonus. Plantar responses flexor. Sensation and general examination normal. A.E.G. demonstrated mild cerebral atrophy. E.E.G. showed abnormality of left fronto-temporal region. C.S.F. protein 40 mg/100 ml. No cells.

Neuro-otological findings

Bilateral paresis of adduction of vocal cords during swallowing and attempted vocalisation. Hearing normal for forced whisper. Optokinetic nystagmus (small drum) responses very feeble. Caloric responses (Fitzgerald and Hallpike) within normal limits. Electro-nystagmographic examination (large drum) optokinetic nystagmus present but eyes deviated in direction of slow component.

Comments and diagnosis

Progressive degenerative neurological disease with dementia and bulbar palsy Abnormality of optokinetic nystagmus indicated some derangement of fast component mechanism, possibly due to a lesion of the frontal centres for voluntary gaze or the projecting fibres therefrom.

Progress

Patient died 3 months later

Autopsy

Findings were reported upon as follows: "The changes in the pre-central cortex and spinal cord are similar to those seen in amyotrophic lateral sclerosis. The changes in the amygdaloid occipital, fronto-parietal and posterior central cortex are indicative of nerve cell loss and gliosis. They are definite and most marked in the region of the posterior frontal depression, which was visible to the naked eye. They are diffuse, making clinico-pathological correlation difficult. The nature of the process seems to be of the Jakob Creutzfeldt group"

Case 4 M M (N H A49249)

A man of 35 under the care of Dr Ross Russell presented with 7 years history of repeated cerebro-vascular accidents and a residual spastic left hemiparesis and dysphagia and anarthria. The last episode had been 4 years ago. He had difficulty in concentration and was emotionally labile

Neurological findings

Anarthric but co-operative and able to communicate by writing. Able to read and type. Fundi, fields and pupils normal. Pseudo-bulbar palsy for cranial nerves 9–12. Hypertonic limbs and residual left hemiparesis. Plantar responses extensor

Neuro-otological findings

He was able to stand and walk unsteadily with the eyes covered. He was unable to keep the eyes closed. Eye movements were as follows.

Command and following movements were full to left and right, up and down.

Convergence normal.

Dolls head movements complete to left and right up and down.

Spontaneous and positional nystagmus absent.

Optokinetic nystagmus (small drum) responses brisk and equal left and right, present up and down.

Caloric test, Fitzgerald and Hallpike technique: brisk responses, slight directional preponderance to left.

Electro-nystagmography (large drum) well marked optokinetic nystagmus was elicited but change in direction of nystagmus on drum reversal took place with a change in direction of slow component and eyes tended to deviate in direction of slow component (Fig. 8)

Comments and diagnosis

Multiple cerebral emboli with residual pseudobulbar palsy spastic limbs and left hemiplegia. Abnormality of optokinetic nystagmus suggested bilateral elimination of pathways subserving fast component of nystagmus controlled by frontal visual centres.

Progress

Treated by anticoagulants. Reported 1 year later to be resident in a mental hospital.

ACKNOWLEDGMENTS

Acknowledgments are due to the physicians of the National Hospital, Queen Square, who have referred their patients for examination and to Professor William Blackwood for the autopsy examination of case L J

RÉSUMÉ

Des études pratiquées sur des malades atteints de lesions de l'écorce et des ganglions de la base du cerveau ont démontré l'existence de deux mechanisms de nystagmus optocinétique, bien separés; l'un de la phase rapide et l'autre de la phase lente. Les centres se trouvent respectivement dans les écorces frontale et occipitale avec une voie d'association corticale entre elles

ZUSAMMENFASSUNG

Klinische Studien an Patienten mit Läsionen des Cortex und der Basalganglien erbrachten Hinweise auf die Existenz zweier unterschiedlicher Mechanismen für die schnelle und die langsame Phase des optokinetischen Nystagmus mit Zentren im frontalen bzw occipitalen Cortex und einer corticalen Associationsbahn zwischen den zwei Zentren.

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DISCUSSION

T Fukuda. In Japan, optokinetic nystagmus is generally used as a proof of vertigo. Optokinetic stimulation evokes strong rotatory vertigo in every normal adult. I think the vertigo is caused in a reflex manner as just shown in the slides. Should there occur pathological disturbances of a reflex type, the resulting vertigo will be of a non-vestibular origin. I would like to ask a question. What kind of apparatus do you use for the test? I remember that a small cylinder was used for the purpose when I visited Dr Hallpike's department. We use a rotating chamber of 1 m radius as reported several times.

J D Hood (Reply) to Mr *Fukuda.* The optokinetic drum used in our investigations is some 6 ft in diameter It completely encloses the subject and is illuminated from the interior The induced vestibular sensations mentioned by Prof. Fukuda are of particular interest. If the drum is rotated to the left this in turn induces a sensation of turning to the right. The sensation, however can at times be inhibited at will by the subject. If, however the subject is asked to fix his gaze upon a stationary point placed close to the periphery of the rotating drum then optokinetic nystagmus is completely inhibited and in addition the sensation of turning is considerably enhanced and cannot be inhibited at will. It seems clear therefore that the magnitude of the sensation of turning is dependent not upon the nystagmus, which indeed is absent in the presence of optic fixation, but upon the passage across the peripheral retina of the images of the stripes of the drum. As a consequence of this the more closely the eye velocity matches that of the drum, the less will be the sensation of turning.

It is interesting in this respect that given constant drum velocity to left and right, patients exhibiting directional preponderance of optokinetic nystagmus experience comparable directional preponderances in their sensations of turning. Thus in the case of directional preponderance of optokinetic nystagmus, say to the right, the velocity of the slow component of the nystagmus to the right is less than that to the left. Consequently movement of the drum to the right will induce a stronger sensation of turning than that to the left.

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RADIATION TREATMENT OF HUMAN TUMOURS FOLLOWING THE IN VIVO SYNCHRONIZATION OF THE CELL CYCLE

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Abstract During its life the cell passes through a number of developmental stages, an event that is referred to as the cell cycle. I the S-phase of the cycle the DNA is duplicated. All cells of a tissue are statistically distributed over the entire cell cycle and enter into the M phase at different times, so that we get the impression of continuous growth f the tissue.

5-fluor-uracil (FU) inhibits DNA-synthesis reversibly so that the cells accumulate at the beginning of the S-phase. Because of the rapid catabolism of FU the cells pass together through the cell cycle after the blockage is terminated, they will be synchronized.

X-ray beams harm the cells severely in the G₂-phase. Thus synchronized population should be injured very badly by irradiation in this phase.

With our in vivo and in vitro method we were able to demonstrate that the ³H-index of different tissues taken from 40 patients usually increased about 7 hours after FU infusion and was followed by peak of mitotic cells 2 hours later as proof of the synchronized population.

T test whether radiotherapy following synchronization would be successful, we used mice with a carcinoma of the skin induced by benzpyrene. The in this way treated animals showed clearly the lowest tumor growth with *prolongation of life-time* compared with the other groups (controls, FU-treated, radiated and combined treated mice).

Working on the results of the animal experiments we used the synchronization method followed by radiotherapy for the treatment of carcinoma-patients who were in definitely incurable condition. In nearly all cases the treatment could be considered beneficial. Serious side effects have not been observed.

During their life span tissue cells pass through a number of developmental stages, an event that is referred to as the cell cycle (Fig. 1) This cycle is composed of the G1 S- G2 and M phases respectively It is during the G1 phase that the cell exerts its specific function but its duration cannot be precisely determined. The three other phases serve for the preparation of cell division and mitosis. Their respective durations are very similar to one another During the S-phase, the DNA is duplicated and thus the genetic information. After a short additional interval (G2-phase) mitosis is completed.

All cells of the growing fraction of a given tissue are in different developmental stages which are statistically distributed over the entire cell cycle. It is this non-synchronized growth of the cells which gives rise to the impression of a continuous growth of tissues.

There are some phases of the cell cycle, particularly the transition from the G1 to the S-phase and also the G2-phase, that are especially sensitive to radiation. To optimize the result of radiation therapy the largest possible number of cells must simultaneously be in one of these phases. To achieve this end, the cell growth must be synchronized throughout the tissue

The antimetabolite 5-fluoruracil (FU) is known to inhibit reversibly the methylation process from uracil to thymidine It therefore blocks the DNA synthesis (Heidelberger et al., 1960) When DNA-synthesis is blocked all cells that are just about to enter the S-phase must remain indefinitely at this stage of their development. When the blockage is terminated FU catabolises rapidly and the inhibited cells pass altogether like a wave, through the mainder of the cycle (Fig. 2) Cons

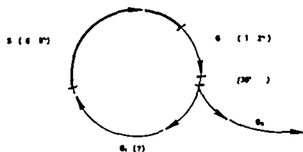


Fig 1 Cell cycle.

the cell populations of the subsequent phases will be increased in number. This increase may be demonstrated by differentiating all cells in their S- and M phase from all others. The number of the M-phase cells are given by the mitotic index as may be read from any histological preparation. The S-phase cells may be recognized by autoradiographic means after having been labelled by ^{3}H-thymidine.

It was upon such considerations that the following regimen was based. patients received 1 0 g of FU in 1 000 ml of 5 4% glucose by infusion during the course of 18 hours. Small pieces of tumour tissue were removed before as well as at different times after the infusion. ter histological preparation the mitotic in- x was determined based upon the valuation of 10 000 cells. The remainder of each specimen was incubated at 37 C for 1 hour in a glass closed by a rubber stopper containing tritiated thymidine in Eagle Medium, and filled up with carbogen This incubation was begun either immediately after the biopsy had been received or 2 to 5 hours later During the latter time, i.e. before labelling, the tissue was incubated in a medium free of ^{3}H thymidine Following a normal autoradiographic procedure the ^{3}H index was determined on a total of 3 000 cells.

Fig. 3 shows a distinct increase of H-labelled cells immediately after the blockage was terminated. The number of labelled cells reached a plateau within about 5 hours and decreased again sharply after 8 hours. After one additional hour i.e. after a total of 9 hours, a distinct increase of the mitosis index could be observed.

For an optimal synchronization effect, 12 hours for infusion are needed (Ganzer & Nitze, 1970 *a*) The time courses of both indices revealed that human tissues may be synchronized in vivo The latter finding together with the fact that the G2 phase is particularly radiosensitive and furthermore our own observation that the S-phase was always of constant duration, suggested that we establish the synchronization of malignant tissue and examine the effect of following-up irradiation. If found successful the same plan would then be pursued in clinical patients.

The main problem encountered in this endeavour was posed by the need to predict the reaction of normal tissues surrounding the neoplasma, as these cells are being synchronized in the same manner as the tumour cells (Fig. 2) However the normal tissues and the malignant ones may be differentiated from either or all of the following points of view



Fig 2 Effect of FU on a non-synchronized growing tissue The cells assembled during the blockage pass altogether like a wave through the remainder of the cycle. G1 S- G M phases of the cell cycle

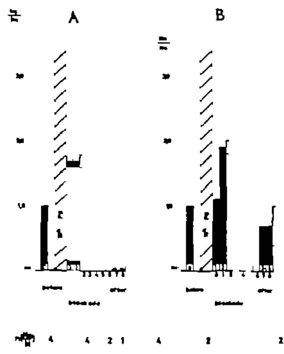
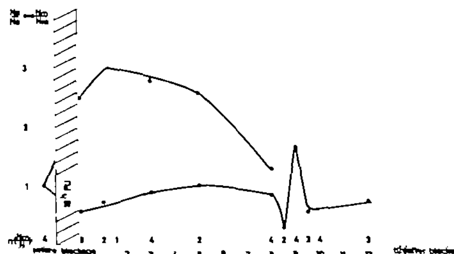


Fig 3 ³H-index of malignant (A) and normal (B) tissues after an infusion during the course of 18 hours as well as the mitotic index (C). Ns/Ns = ³H-index, •—• Nm/Nm = mitotic index, ○—○ = number of tissues.



1 There may be a different growth fraction in normal and malignant tissues

2 These two types of tissue may pass the S-phase at different speeds

3 The maximal radiosensitivity of normal and malignant tissues may not occur during the same phase.

Each of these three hypotheses was examined in separate experiments however no extensive investigations were carried out.

We used mice with skin carcinomata which had been induced by benzpyrene. The animals were divided into five groups: group I was used as a control group group II received FU group III was treated cytostatically with FU and then irradiated on the next day according to the combined treatment as in clinical use today group IV was only irradiated, and, finally in group V the tissue cells were synchronized by repeated subcutaneous injections of FU and they were irradiated precisely 8

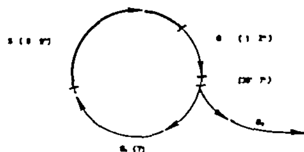


Fig 1 Cell cycle

the cell populations of the subsequent phases will be increased in number This increase may be demonstrated by differentiating all cells in their S- and M phase from all others. The number of the M phase cells are given by the mitotic index as may be read from any histological preparation. The S-phase cells may be recognized by autoradiographic means after having been labelled by ^{3}H-thymidine

It was upon such considerations that the following regimen was based. patients received 1 0 g of FU in 1 000 ml of 5 4% glucose by infusion during the course of 18 hours. Small pieces of tumour tissue were removed before as well as at different times after the infusion. *ter histological preparation the mitotic in*dex was determined based upon the valuation of 10 000 cells. The remainder of each specimen was incubated at 37 C for 1 hour in a glass closed by a rubber stopper containing tritiated thymidine in Eagle Medium, and filled up with carbogen. This incubation was begun either immediately after the biopsy had been received or 2 to 5 hours later During the latter time, i.e. before labelling, the tissue was incubated in a medium free of ^{3}H-thymidine. Following a normal autoradiographic procedure the ^{3}H index was determined on a total of 3 000 cells.

Fig. 3 *shows a distinct increase of ^{3}H la*belled cells immediately after the blockage was terminated. The number of labelled cells reached a plateau within about 5 hours and decreased again sharply after 8 hours. After one additional hour i.e. after a total of 9 hours, a distinct increase of the mitosis index could be observed.

For an optimal synchronization effect, 12 hours for infusion are needed (Ganzer & Nitze, 1970 *a*) The time courses of both indices revealed that human tissues may be synchronized in vivo The latter finding together with the fact that the G2-phase is particularly radiosensitive and furthermore our own observation that the S-phase was always of constant duration, suggested that we establish the synchronization of malignant tissue and examine the effect of following-up irradiation. If found successful the same plan would then be pursued in clinical patients.

The main problem encountered in this endeavour was posed by the need to predict the reaction of normal tissues surrounding the neoplasma, as these cells are being synchronized in the same manner as the tumour cells (Fig. 2) However the normal tissues and the malignant ones may be differentiated from either or all of the following points of view

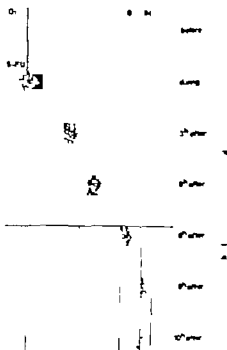


Fig Effect of FU on non-synchronized growing tissue The cells assembled during the blockage pass altogether like wave, through the remainder of the cycle G1 S-, G M phases of the cell cycle.

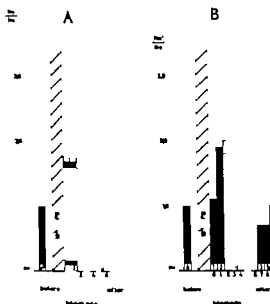
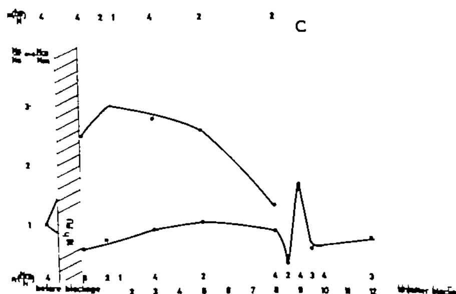


Fig 3 ^{3}H-index of malignant (A) and normal (B) tissues after an infusion during the course of 18 hours as well as the mitotic index (C). Ni /Ni ^{3}H-index, •—• Nm /Nm mitotic index, o—o number of tissues.



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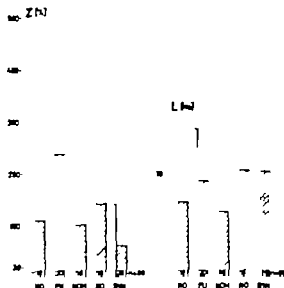


Fig 4 Increase of tumour growth and survival time of mice with skin carcinomata treated in different ways. *KO* control group *FU* cytostatically treated animals, *KOM* combined treatment, *RO* irradiated mice *SYN* synchronization followed by radiation treatment. Z. growth of tumour L. survival time

hours after the end of the blockage All animals of groups II III and V received the same dosage of FU (4 times 0.5 g subcutaneously) and the same amount of X ray irradiation oups II IV and V 4 times 500 rad aoul's technique) The results are shown in Fig. 4 The survival times of the animals in the group which had received the combined treatment (group III) and that of those belonging to the control group (group I) were similar to each other but relatively short. The cytostatically treated animals (group II) survived definitely longer and the irradiated and the synchronized group of mice (group V) survived for the longest periods. The "synchronized" group of mice showed unequivocally the lowest rate of tumour growth with a clear extension of their life span. Although the irradiated animals (group IV) survived for nearly the same length of time, their tumours had grown over this period to one-and a-half times the size of the tumours found in the synchronized animals. The high standard deviations, especially of the growth rate were most likely due to the use of autochthonously growing tumours instead of transplanted ones (Ganzer & Nitze 1970 *b*)

Encouraged by these findings we have started to apply the method of synchronization with subsequent radiotherapy to the treatment of cancer patients. At the time of this writing, 13 patients have undergone this type of treatment. Nearly all of them had had previous surgery or had received radiotherapy or both. Further surgery or radiotherapy was deemed inadvisable owing to the size or the location of the tumour and to the general condition of these patients. In some cases the treatment *given to the patients had consisted only of synchronization* followed by radiotherapy All these patients were in an absolutely incurable condition. The present treatment consisted of infusion with 1 0 g FU in 1 000 ml of 5 4% glucose lasting for exactly 12 hours and radiotherapy with fast electrons (betatron) of 500 rad given precisely 8 hours after termination of the infusion This combined treatment was given to the patients twice a week until a total dosage of 4 000 to 6 000 rad was reached. Side effects have been observed in only a few cases these were nausea and vomiting. Red and white blood cells and thrombocytes were examined once a week, but they did not show any decrease to critical pathological levels. In all cases the treatment could be considered beneficial The tumours always showed considerable regression especially those situated at the free surface of the cavity of the mouth. It became evident that the chance of removing the destroyed tumour as quickly as possible may

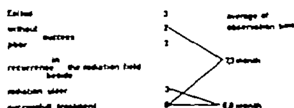


Fig 5 Result of 13 patients treated with the synchronization method.

also play an important role. Tumours situated directly under the skin showed a clear decline in their growth rate even as long as 8 weeks after treatment.

Fig. 5 summarizes the results. In 2 of 3 cases the treatment came too late patients were in generally poor conditions, their tumours being extremely large. One patient died as a result of a tumour recurrence after having received synchronization therapy and only 4 000 rad, she refused a second treatment. The ulcers induced by the radiation and found in 3 of 8 cases demonstrate the sensitivity to radiation of the synchronized normal tissue. However in the majority of cases there was a clear difference in radiosensitivity between normal cell tissues and malignant ones since only malignant tissue was destroyed or injured, but not the normal tissue.

This is the *first* time that cell-kinetic parameters are being considered in the radiation treatment of cancer It goes without saying that the method is not yet perfected. It will be improved if and when further experiments and investigations on cell-kinetics present new insights. However we feel that the synchronization treatment followed by radiotherapy can already be used in the clinical management of human cancer aiding in our effort to find more successful ways in the treatment of cancer

ACKNOWLEDGMENT

W thank for their kind cooperation Prof. Dr W Lorenz, head of the Universitätsklinik für Strahlentherapie und Nuklearmedizin, Frankfurt/M and Prof. Dr D Schmähl, head of the Institut für experimentelle Toxikologie und Chemotherapie am Deutschen Krebsforschungszentrum, Heidelberg.

RESUMÉ

Toutes les cellules d'une population en croissance sont indépendantes les unes des autres à ce qui concerne la prolifération. Pendant les différentes phases de leur développement change la radiosensibilité de la cellule. Accumulant les cellules par une infusion de FU dans la phase la plus radiosensitive avant de les irradier, l'efficacité d'une radiothérapie doit être améliorée. Les résultats de recherches expérimentelles et cliniques montrent la superiorité de ce procédé.

ZUSAMMENFASSUNG

In den verschiedenen Entwicklungsphasen des Zellzyklus wechselt die Strahlensensibilität der einzelnen Zelle. Werden durch eine 5-FU Dauerinfusion Zellen in der strahlensensiblen Phase angesammelt und hier bestrahlt, müsste sich der therapeutische Strahleneffekt verbessern lassen. Tierexperimentelle Untersuchungen und die folgende Anwendung in der Klinik bestätigen die Richtigkeit dieser Überlegung.

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DISCUSSION

Ch. Beck. Wir haben in den letzten Monaten drei Patienten mit inkurablen malignen Tumoren der Zunge und des Hypopharynx mit der Synchronisation behandelt. Bei allen ist der Tumor verschwunden.

L. B W Jongkees I am not yet convinced by the statistics of M Vosteen or by the cases he showed. I have seen too many cases that reacted marvellously to chemical treatment alone, both given via catheter with temporal arteries and as general treatment. Most of them, however are only temporary results

K.-H Vosteen (Reply) Die Synchronisationstherapie menschlicher Tumoren ist nur die logische Konsequenz von experimentellen Ergebnissen unserer Beschäftigung mit den theoretischen Problemen der Zellkinetik. Das wichtigste Resultat war dass es überhaupt gelingt, menschliches Gewebe in vivo zu synchronem Wachstum zu zwingen. Die Überlegenheit der Synchronisationstherapie gegenüber Bestrahlung oder radiologisch-zytostatischer Behandlung in üblicher Kombination ergibt sich eindeutig aus den beschriebenen Tierversuchen. Natürlich sind die Therapieerfolge noch zu frisch, um sie endgültig beurteilen zu können. Man muss aber berücksichtigen, dass es sich bei allen Patienten um "inkurable Fälle in verzweifelter Situation gehandelt hat. Deshalb ist eine durchschnittliche rezidivfreie Über' 7 Monaten schon al Erfolg anzusehen.

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COCHLEAR MASKING AND ADAPTATION

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Abstract Intracochlear electrodes were used to measure the action potentials (AP) generated by successive clicks. During a series of clicks the AP decreases in a specific way This decrease is called by many investigators adaptation of central origin. It is influenced by repetition rate intensity and masking by noise or tone. It was found that adaptation and readaptation are controlled by the positive after-potential generated by a click or a tone or noise burst. Also a positive phenomenon like the +SP could influence the magnitude of the AP It is postulated that this fast adaptation is influenced by a positive DC potential in the vicinity of the hair-cells, and thus must be of cochlear origin. A long-term adaptation due to our low intensity stimulus could not be demonstrated. It is possible that peripheral (fast) and central (slow) adaptation act in the same way on different intensity levels, by causing a +DC potential around the hair-cells.

When a click, acoustically consisting of a wide and spectrum, is offered to the ear of an animal, a volley of action potentials (AP) can be detected inside the cochlea. This AP is a synchronised summation of action potentials fired by a large number of hair-cells resulting in a broad electrical phenomenon of negative polarity with a latency of approx. 1 msec and a duration of approx. 2 msec. This AP consists of two parts, N_1 and N_2, which is a second negative potential coming approx. 1 msec after N_1 N_2 is followed by a positive after potential. The AP can be considered as a neural overall answer of the cochlea to a well defined acoustical stimulus. This AP has lost the interest of workers dealing with neural behaviour of the cochlea. It was overtaken by the interest in the single fibre of the acoustic nerve However to be informed about the total cochlear response to an acoustical stimulus, not only the single unit in the cochlea is of interest but also the co-operation of the different single fibres resulting in the AP The AP can be influenced by many parameters such as intensity of the click (Deatherage et al., 1959) a click interval time (Peake et al., 1962) rarefaction and condensation (Peake et al., 1962) and probably the most significant one masking by tone or noise (Coats, 1964 1969 Teas et al., 1962 Teas & Gretschen, 1969) All these influences can be measured as a reduction of magnitude of AP a change in latency of N_1 and a variation in duration of the AP A biological condition accompanied by reduction of AP magnitude without metabolic dysfunction is adaptation. Thus adaptation can be of central (Galambos, 1956 Fex, 1962) or of peripheral origin (Keidel, 1961) When we are dealing with adaptation of central origin exerted by the Rasmussen bundle we are informed by the work of Galambos (1956) who found a suppression of the AP during electrical stimulation of this olivo-cochlear bundle and by the work of Fex (1967) who found that the same stimulation gave a change in the DC potential in the scala media acting as a regulator on the firing of AP Central influence on cochlear function has been discussed recently by Pfalz (1969) who pointed out that under physiological conditions the central efferent influence on cochlea is negligible. The hypothesis is formulated by Fex (1967) that the uncrossed efferent fibres may also in-

These investigations were supported in part by a grant from the Netherlands Organisation for the Advancement of Pure Research. (Z.W O)

teract directly on the afferent terminals underneath the outer hair-cells. Measurements of Whitfield (1967) suggest that the fibre distribution as it is found inside the cochlea could be held responsible for a peripheral fast adaptation inside the cochlea. When we want to decide about regulatory mechanisms on the bioelectrical output of the cochlea, two main systems are available: the crossed and the uncrossed system. The uncrossed system can interact on almost any level from nucleus cochlearis to the post-synaptic connections inside the organ of Corti. In the following experiments an attempt is made to separate adaptation of central origin from fast peripheral adaptation.

METHOD

Healthy guinea pigs with an average weight of 350 g were anaesthetized with a 1 10 Nembutal-Urethane Mixture 300 mg/kg body weight. In all experiments a tracheostoma was performed to maintain a free air passage. The cochlea was exposed from the laryngeal side, after opening of the bulla. In several animals the function of the middle ear musculature was eliminated by cutting the tendons. Silver/silver-chloride electrodes with a diameter of 50 μ were introduced into the turns of the cochlea by means of bore holes in scala tympani. The animal was fixated in a headholder with a built-in metal cannula that was inserted into the outer ear-canal a condensor test microphone and a specially made condensor-telephone receiver with a flat response from 60–30 000 Hz were connected to it. The electrodes were fed into a low noise differential pre-amplifier (amplification × 1 000) from here the signal was conducted to a four channel oscilloscope and to an averager CAT 1 000 After summation the graphs were portrayed on a Moseley X–Y recorder To produce the complex mixture of clicks and masking tones or noise that was offered to the animal, six electronically gated switches were used. they could switch sequentially or partly in parallel. Every switch produced a pulse so a function generator could be triggered with the opening of the switch. In this way a masking tone could be synchronized. The last one of the six switches could trigger the first so that a recycling generator with a constant repetition frequency was formed. This electrical tone burst was fed into a power amplifier that delivered also the +200 V polarising voltage for the condensor-telephone receiver in use. Each click had a duration of 100 μsec. Acoustically measured it was a fast damped transient with a duration of 500 μsec so the sound spectrum covered a wide frequency range. The intensity was measured re 0 0002 dyns/cm² sound pressure level SPL.

RESULTS

1 *General remarks*

The AP generated by a click was measured in different turns of the cochlea by inserting an electrode into the scala tympani. There was no measurable difference in appearance of the AP which turn was chosen so far The major part of the AP was the N_1 which represented the synchronized activity of thousands of active neural elements. When, in a descending series of measurements, the intensity of the click stimulus reached an intensity of about 20 dB above threshold, N_2 started to fade away At threshold levels only N_1 could be measured. N and N were always followed by a slow positive potential (P) with a duration related to the magnitude of N_1 (Fig. 1) The total duration

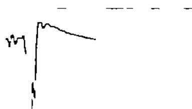


Fig 1 The summed action potential AP N N and positive after-potential P are clearly demonstrated after summing up (100) (Stimulus: 100 μsec click with an intensity of 50 dB SPL.)

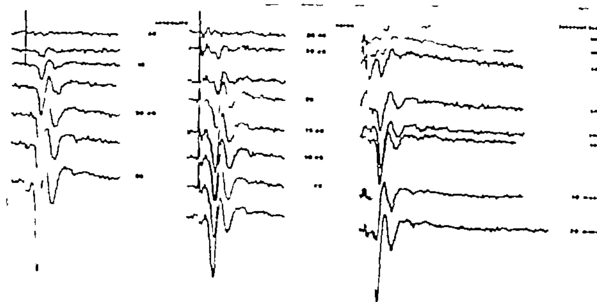


Fig 2 The influence of intensity repetition rate and masking on AP Note the identical latency shift between 1.5 msec and 1 msec and the similar input-output behaviour

of AP including *P* could reach up to 40 msec at the highest intensity levels used in our experiments (60 dB SPL) The AP was preceded by cochlear microphonics (CM) This was a reliable indicator (when measured in the first turn) of the temporal start of the movement of the cochlear partition. The latency of N_1 was ...red from this point. Masking consisted ... wide band noise pass and noise and pure tone bursts of variable duration, frequency and intensity Of course this masking acted itself as a stimulus followed by a response with an on and off AP a Summating Potential (SP) and a CM Conditions were chosen in such a way that interferences were avoided as much as possible

2. *The influence of repetition rate intensity and masking on AP*

These measurements were merely performed to get connection with work done by others, so this will be only a brief summary of results. The repetition rate of the click was varied from 10/sec to 3 000 sec both amplitude and latency of the AP changed and that in such a way that the latency increased and the amplitude decreased. The same effects could be obtained by reduction of the intensity from 50 dB to threshold. Also measurements were performed by masking with a wide band noise of the AP generated by a 50 dB SPL intensity click. When the intensity of the noise was increased the amplitude of the AP decreased and the latency shifted to a longer time These three forms of AP behaviour are portrayed together in Fig. 2. It is shown, that difference in latency between smallest and largest AP always will be approx. 0 6 msec under all three conditions.

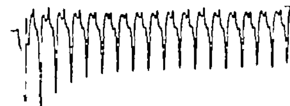


Fig 3 The adaptation as measured with successive click having an interval of 3 msec and an intensity of 50 dB SPL

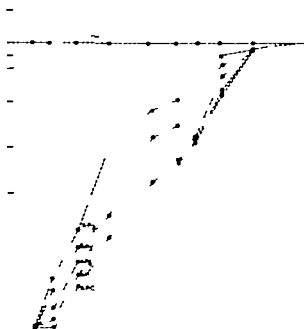


Fig 4 The influence of the interval time of the clicks on the successive AP' (AP_1–AP).

3 *The influence of successive clicks on the generated AP*

When clicks are offered in a successive mode the ensuing AP's will show a specific behaviour The first AP has the largest magnitude, the following AP always will be smaller than the preceding one (Fig. 3) *After a number of* clicks a steady state of the AP is reached and no further change in amplitude or form will take place. The influence from one AP on the following one is related to two main factors: interval time and intensity of the clicks. First of all the influence of the interval time on the magnitude of the AP_2, AP_3, AP was measured. This AP behaviour we will call adaptation. It was found that the AP will become smaller in magnitude when the interval time is made shorter. If the interval is so short that one N_1 will mingle with the preceding N_2 (interval time <2 msec) AP will already have reached the constant value of AP (Fig. 4) With a long time interval exceeding 50 msec AP $= AP_2 = AP_3 =$ AP while at a very short interval time AP $=$ AP $=$ AP (AP $> AP_2$) Intensity has another influence on adaptational behaviour With interval time kept constant (in this case 3 msec) the relation between successive AP's was measured. When the intensity increased the AP-curves spreaded but the relationship between AP and AP stayed constant (Fig. 5)

4 *Masking by tone and noise of the successive AP's*

A. *Simultaneous masking* 3 msec interval clicks were offered to the guinea pig ear By means of the complex switching and time device a masker (tone or noise) could be introduced during bursts of clicks. After about 30 msec of clicks, the masker was added for 30 msec, followed by 40 msec of clicks alone in order to study recovery of AP's after the masking. We will call this recovery readaptation. When a tone was introduced the generated CM

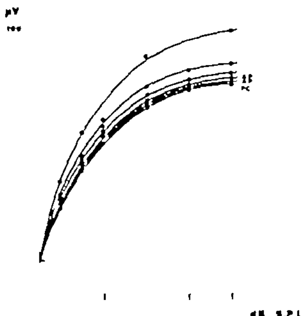


Fig 5 The input-output function of successive AP's; note that at low intensity levels the adaption is quite moderate.

would interfere with the AP s under measure. *Therefore the tone was non-synchronous with* the start of the clicks after 100 averages with the CAT 1000 the CM was cancelled and the AP's due to the offered clicks were clearly re gistered on the screen of the CAT 1000 This ethod is more favourable than filtering be use filter-distortion is avoided. First of all tone variable in frequency was used as a masker The intensity was chosen in a fixed relation to the offered clicks, namely a masker to-click amplitude ratio of 2 to 1 measured in the electronic representation of the total signal on the screen of the oscilloscope. When the frequency of the tonal masker was changed it was found that there was a decrease of the AP s on a specific frequency When the total stimulus (that is, masker and click in constant ratio) was measured with intensity as a parameter a remarkable change in optimum mask ing frequency was seen, it shifted to a lower part of the frequency range, that ranged from 3 000–7 000 Hz. In the following series of measurements the tonal masker was exchanged for a noise masker Because of the wide frequency spectrum the masker intensity had to be decreased to avoid over-masking. When the intensity of the total stimulus (noise and clicks) was increased the AP amplitude during mask ing stayed constant at the level at which mask ing first was noticeable A decrease of intensity below this level diminished both masked and unmasked AP in the same relation (Fig. 6).

B *Previous masking* In this set-up the noise masker was started already 1 000 100 and 10 msec before the clicks were offered Now re adaptation and long-term recovery of AP could be measured separately It was found that there was no influence on recovery of AP during 100 msec after readaptation was completed, related to the duration of the previous noise. The readaptation was not influenced at all whether the previous masking time was 10, 100 or 1 000 msec. The previous masking did not exceed *an intensity of 60 dB SPL.*

Relation between masking of AP and DC potentials

The +SP and −SP have already been de scribed in previous work (Kupperman, 1966, 1969) but a short review will be given here The +SP can be measured in that place in the cochlea (scala tympani) where cochlear partition shows maximum movement during stimulation with a tone burst. In all other parts a −SP will be found in scala tympani. In the following experiments the masking tone burst as described before will serve as a gener ator for −SP and +SP as well. To obtain this condition a tone burst with a frequency of 5.5 kc with an intensity twice the intensity of the clicks and a duration of 50 msec was offered 20 msec after the start of the clicks. Simultaneously in the third and first cochlear turns the bio-electrical potentials were measured. The phase of the sinus were not synchronized so its *CM was cancelled on averaging. In* the first turn a +SP was generated and in the third turn a −SP was found. Still the tone burst acted as a masker and the AP's were of course diminished in amplitude, but it was clearly demonstrated (Fig. 7) that during gener

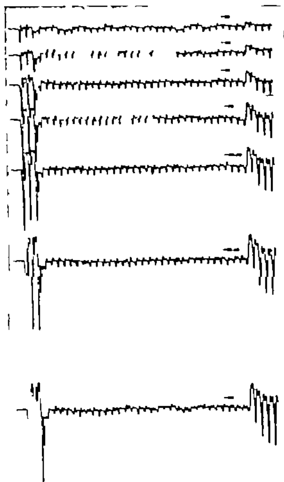


Fig. 6 Masking by noise burst of the AP Increasing the intensity of noise and clicks does not influence the magnitude of the masked AP. Note the readaptation after the tone burst and its dependence on the intensity of the noise burst.

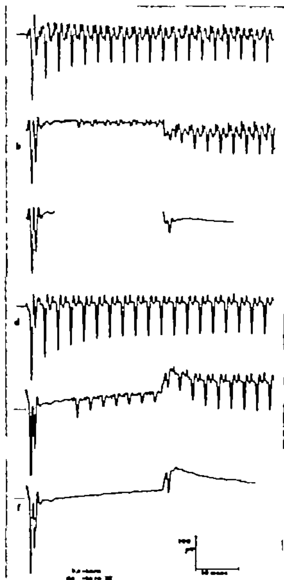


Fig. 7 The influence of +SP and −SP on AP. The tone burst has a frequency of 5.5 kc. Clearly is demonstrated that during the existence of the +SP in the basal turn the AP is decreased to smaller magnitude than in the 3rd turn where −SP is found. Note that without the masker the findings in the first and third turn are identical.

ation of the +SP there was a smaller amplitude of the AP's than during the −SP in the third turn. When there was no masker present there was no difference between the AP's measured in third and first turn. So a positive DC potential measured in the scala tympani is related to a maximum masking effect of the AP related to the click.

Another positive potential is already demonstrated in Fig. 1 where a positive after-potential is portrayed. To study the influence of this potential in a click, two clicks are offered to the guinea pig ear the time between the clicks is varied. It was found that exceeding an

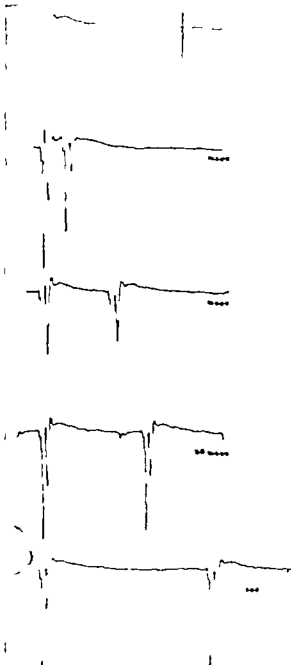


Fig. 8. The influence of the positive after-potential *P* of an AP on the following AP. Only when the *P* has disappeared will the second AP reach the normal value (intensity is 50 dB SPL).

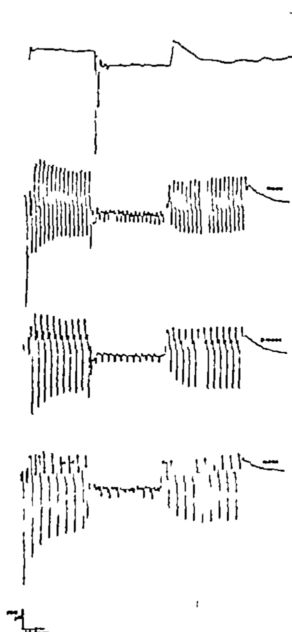


Fig. 9. Readaptation measured after a burst of noise clearly demonstrates that the duration of readaptation is identical with the duration of the after potential generated by the noise.

interval time of 40 msec the first AP does not interfere with the second click. Otherwise the AP_2 was smaller than the preceding AP (Fig. 5). The duration of the after potential was also 40 msec: this corresponded with the interference time of the two AP's (Fig. 8). Not only can a click generate the positive after potential but also a burst of noise (in a tone burst more complex effects happen (Fig. 9)). When we compare the time of readaptation using clicks of different interval time and the duration of

the positive after potential following the noise burst then we will find that these two times will correspond. The readaptation occurs during the presence of the positive after-potential.

DISCUSSION AND CONCLUSIONS

In order to collect more information about cochlear adaptation in the guinea pig, measurements are performed in which the influence of repetition rate, intensity and masking of clicks on AP magnitude is determined. The measurements can be compared if the threshold of AP generation is used as a starting point. All these measurements have in common that within certain limits the influences of intensity change, repetition rate and masking on the magnitude of the AP are similar. If we consider the AP to be built up as the product of different active neural elements inside the cochlea, the only conclusion that can be drawn from these findings is that there is an alteration in synchronisation in the case of masking, a change in the number of total active elements in the case of intensity change and a combination of these two conditions in the case of repetition rate. But the change in magnitude is not the only effect. A more important phenomenon is the change in latency of the AP (measured as time between the CM and the N_1 of the AP). The latency increase always occurs when the magnitude of the AP is reduced, whether this is due to intensity decrease, increase of repetition rate, or masking by tone or noise. Coats (1969) made some suggestions about the origin of the latency change and arrived at the conclusion that it must be due to a retarding of a hypothetical cochlear excitatory process. This retarding could not be valid for the case of the repetition rate influence while in that instance a maximum number of hair-cells is active in the case of a high repetition rate and still there is an increase in latency. So not only is the excitatory process time of influence but also the synchronisation of the firing of the active elements. Adaptation as it was found by Galambos (1956) and by Desmedt & Monaco (1962) consisted of a suppression of the AP after electrical stimulation of the olivo-cochlear bundle in the floor of the fourth ventricle. This form of adaptation had a certain long-term character, it took about 75 msec to be fully developed and lasted for about 70 msec afterwards. But without this electrical stimulation which has some unphysiological risks, it is possible to find a form of adaptation that is related to the cochlea purely. This is the typical decrease in magnitude of the AP during the stimulation of the cochlea with successive clicks. As has been demonstrated in our experiments the decrease in amplitude is strictly related to the interval time of the successive clicks. After an interval of approx. 50 msec was exceeded, no influence on the magnitude of the AP was measured but under 2 msec interval the magnitude of AP suddenly decreased. It was not possible to decide whether we were dealing with an AP or a CM. Below an interval of 2 msec the successive AP's disturb each other strongly and the decrease in amplitude could be on the account of the refractory period. Above an interval of 2 msec another influence must be held responsible for the decrease in amplitude of the successive AP's.

Let us first consider the experiments performed by Fex (1967) who found that there is a relation between electrical stimulation of the crossed olivo-cochlear bundle and a change of the positive DC potential in the scala media, even to a negative value. He concluded that this represented postsynaptic activity generated by crossed olivo-cochlear fibres connected to outer hair-cells. These findings suggest that also during normal stimulation a DC potential can be measured in the vicinity of the hair cells if also a form of adaptation is present.

Experiments of Konishi et al. (1970) point in the same direction. They measured the influence of a small current applied between scala tympani and scala vestibuli on the impulse discharges of the primary auditory fibres. They found adaptation of the nerve impulses and suggested that the excitability of the hair

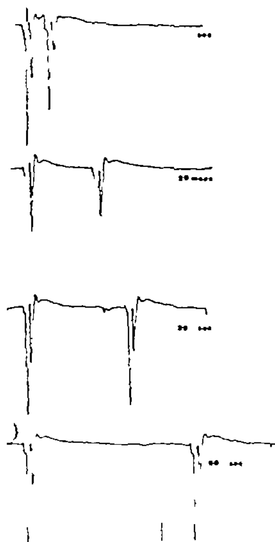


Fig. 8. The influence of the positive after-potential *P* of an AP on the following AP. Only when the *P* has disappeared will the second AP reach the normal value (intensity is 50 dB SPL).

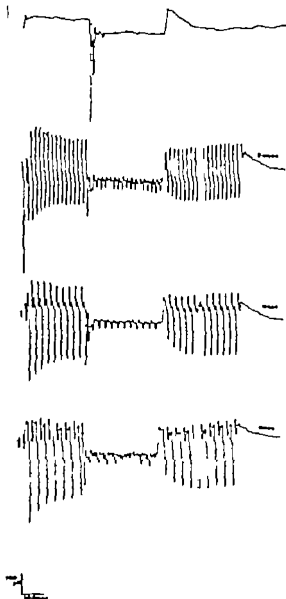


Fig. 9. Readaptation measured after burst of noise, clearly demonstrates that the duration of readaptation is identical with the duration of the after potential generated by the noise.

interval time of 40 msec the first AP does not interfere with the second click. Otherwise the AP_2 was smaller than the preceding AP_1 (Fig. 5). The duration of the after potential was also 40 msec; thus corresponded with the interference time of the two APs (Fig. 8). Not only can a click generate the positive after potential but also a burst of noise (in a tone burst more complex effects happen (Fig. 9)). When we compare the time of readaptation using clicks of different interval time and the duration of

the positive after potential following the noise burst then we will find that these two times will correspond. The readaptation occurs during the presence of the positive after potential.

DISCUSSION AND CONCLUSIONS

In order to collect more information about cochlear adaptation in the guinea pig, measurements are performed in which the influence of repetition rate, intensity and masking of clicks on AP magnitude is determined. The measurements can be compared if the threshold of AP generation is used as a starting point. All these measurements have in common that within certain limits the influences of intensity change, repetition rate and masking on the magnitude of the AP are similar. If we consider the AP to be built up as the product of different active neural elements inside the cochlea, the only conclusion that can be drawn from these findings is that there is an alteration in synchronisation in the case of masking, a change in the number of total active elements in the case of intensity change and a combination of these two conditions in the case of repetition rate. But the change in magnitude is not the only effect. A more important phenomenon is the change in latency of the AP (measured as time between the CM and the N of the AP). The latency increase always occurs when the magnitude of the AP is reduced, whether this is due to intensity decrease, increase of repetition rate or masking by tone or noise. Coats (1969) made some suggestions about the origin of the latency change and arrived at the conclusion that it must be due to a retarding of a hypothetical cochlear excitatory process. This retarding could not be valid for the case of the repetition rate influence while in that instance a maximum number of hair-cells is active in the case of a high repetition rate and still there is an increase in latency. So not only is the excitatory process time of influence but also the synchronisation of the firing of the active elements. Adaptation as it was found by Galambos (1956) and by Desmedt & Monaco (1962) consisted of a suppression of the AP after electrical stimulation of the olivo-cochlear bundle in the floor of the fourth ventricle. This form of adaptation had a certain long-term character: it took about 75 msec to be fully developed and lasted for about 70 msec afterwards. But without this electrical stimulation which has some unphysiological risks, it is possible to find a form of adaptation that is related to the cochlea purely. This is the typical decrease in magnitude of the AP during the stimulation of the cochlea with successive clicks. As has been demonstrated in our experiments the decrease in amplitude is strictly related to the interval time of the successive clicks. After an interval of approx. 50 msec was exceeded, no influence on the magnitude of the AP was measured, but under 2 msec interval the magnitude of AP suddenly decreased. It was not possible to decide whether we were dealing with an AP or a CM. Below an interval of 2 msec the successive AP's disturb each other strongly and the decrease in amplitude could be on the account of the refractory period. Above an interval of 2 msec another influence must be held responsible for the decrease in amplitude of the successive AP's.

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Experiments of Konishi et al. (1970) p[oint] in the same direction. They measured the in[-] fluence of a small current applied betw[een] scala tympani and scala vestibuli on the [im-] pulse discharges of the primary auditory [fibres]. They found adaptation of the [...] [...] and suggested that the excitabi[lity]

cell was influenced by variation of the resting current through the hair-cell. Now let us return to our experiments. We are dealing with a certain behaviour of the successive AP's in the cochlea which is called adaptation. Some authors suggested this phenomenon to be of central origin (Leibbrandt, 1965) others (Spoor 1965 Rodenburg, 1967) found arguments for a peripheral explanation because after careful sectioning of N VIII adaptation was still there In our experiments we tried, only by offering low level physiological stimuli, to decide whether the adaptation is of central or peripheral origin and on which intensity level it occurs. If we compare the influence of repetition rate on AP and the steady state after adaptation, in our experiments called AP_n, then we are dealing with the same AP The collection of the behaviour of the single AP and the adapted AP has already been discussed so that we have here an argument for a peripheral place of origin of AP adaptation. We also measured the influence of one AP on the following AP and found that adaptation occurred only if the after potential generated by the first click was still measurable This also suggests, because this after potential as a DC phenomenon is strictly related to the cochlea, at this adaptation must be peripheral. The fluence of +SP on the successive AP's gives us another argument. The +SP is measured in scala tympani and as a +DC phenomenon it is strictly related to that place in the cochlea which is in maximum movement. The hypothesis is made (Kupperman, 1966) that a leakage from the scala media through the hair-cells during stimulation is responsible for the +SP The vicinity of the hair-cells is now a positive field which can be held responsible for the adaptation of a great number of hair-cells. These findings are in good agreement with these of the other authors mentioned. We also tried to influence successive AP's after prolonged masking, but keeping the intensity under the value of 60 dB SPL hardly any influence could be found on the successive AP's after the positive after-potential had faded away

The conclusions can be made that:

1 Fast adaptation arises at low level stimulation and is of cochlear origin.

2. Slow adaptation comes in at much higher intensity levels exceeding 60 dB SPL and can be of central origin.

3 Cochlear adaptation is related to a +DC shift in scala tympani underneath the hair-cells.

4 There is a possibility that central and peripheral adaptation act by a +DC chance in the vicinity of the hair-cells.

RESUMÉ

Effets de masque et d'adaptation des décharges de potentiels d'action (AP) successives générées par des cliques presentés à l'organe de l'ouie du cobaye sont mesurés comme fonction de different types de masque et d'adaptation, semblent être identiques. Cette mode d'adaptation périphériques peut être distinguée de l'adaptation efferente et produit ainsi une indice importante de la fonction cochléaire

ZUSAMMENFASSUNG

Intracochleäre Elektroden wurden verwendet um das Summenaktionspotential (AP) zu messen, generiert von aufeinanderfolgenden Klicks. Während einer Klickreihe verringert die AP in einer spezifischer Weise. Diese Verringerung wird von manchen Autoren Adaptation von zentraler Herkunft genannt. Sie wird beeinflüsst von Wiederholungsfrequenz, Intensität und Maskierung, entweder von Geräusch oder Ton. Es wurde gefunden dass Adaptation und Readaptation unter Einfluss stehen vom positiven Nach potential, hervorgebracht vom Klick, Ton oder Geräusch. Auch konnte ein anderes positives Phenomen wie der +SP die AP-Grösse beinflussen. Es wird vorgeschlagen, dass diese schnelle Adaptation verursacht wird von einem Gleichstrompotential in der Nahe der Haarzellen sie muss also von Kochleären Herkunft sein. Eine langdaurige Adaptation bei unserem niedrigen Schallpegel wurde nicht gefunden. Es wird möglich gehalten dass periphere (schnelle) und zentrale (langsame) Adaptation dieselbe Wirkung haben bei verschiedenen Schallintensitäten, durch Veranlassung eines positiven Gleichstrompotentials rund den Haarzellen

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DISCUSSION

H Davis. M Kupperman has given beautiful description f both action potentials and other slow potentials. The latter are still poorly understood and probably complex in origin. To what extent does the second phase of the action potential contribute to the positive afterpotential? What do you believ to be the general biologic contribution of the olivocochlear efferent system?

J Tonndorf: Mr Kupperman showed difference in the noise response between 1st and 3rd turns H changed this difference to the well-known opposite polarity f the SP between 1st and 3rd turns. There is another possible explanation, and that may have to do with the fact that in response to clicks (noise) the 3rd turn response is usually longer than that on the 1st turn. I don't know how one could decide this question experimentally

J David. Is there an adaptation-effect when avoiding the influence of the efferent system for example by cutting the VIII nerve?

R. Kupperman (Reply) to Mr Davis I compared the positive afterpotential after burst of noise with a single action potential and found that there was only slight difference in duration. This could not be due to second phase of the afterpotential. The general biological value of the decrease of the AP' during cochlear adaptation can be increased by not referring to the magnitude of the AP but to the total area of the AP so that square function is obtained. W were not able to find central influence on the magnitude of the AP Of course w could not use any method other than the measurement of the successive AP because we have to compare t the total cochlear behaviour where we need the AP

To M *Tonndorf* T obtain an AP with opposite polarities in the different turns of the cochlea w used 5 5 tone burst to cancel out the microphonics. W averaged the non-synchronous cochlear microphonics

To M *David.* Sectioning of the VIII nerve was already performed by Rodenburg. There was no influence on the behaviour of the decrease of the successive AP's.

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D C. POTENTIALS IN THE AUDITORY EVOKED RESPONSE IN MAN

W D Keidel

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Abstract. While the conventional auditory evoked response is mainly related to the onset of auditory stimuli and/or to their cessation (off-effect) during prolonged sinusoidal stimulation, a marked d.c. potential could be recorded by means of a special d.c.-recording set-up in both animals and in man. Here, special precautions had to be taken to avoid polarization—artefacts originated by the electrodes (Beckman-type). Those difficulties have been overcome and clearly reproducible records of d.c.-potentials in man could be obtained in our Institute. These d.c.-potentials depend upon both stimulus intensity and stimulus duration. They show in addition interaction with other sensory modalities such as visual ones. A comparison of their time course with interval and PST-histograms from single units in colliculus and geniculate prove their intramodal specific nature. The importance of the d c.-potential for the objective audiometry is demonstrated.

When we deliver a continuous tone or a very rt sound like a click or a brief tone burst /un animal s, or a human ear the cortical responses to these auditory stimuli are actually not very different when recorded with our conventional technique. In any case we observe a marked response to the onset of the stimulation, the on-effect, and a similar but smaller response at the end of the stimulus, the off effect. When the duration of the tone as a stimulus is sufficiently long, both the responses described can be distinguished clearly In the case of tone bursts, or brief tones such as clicks, any type of interaction between the on- and off-effect may result. Recording with the established technique of objective audiometry" however it is usually not possible to detect any response during the ongoing prolonged stimulus. That there are exceptions, we want to show in this paper and those exceptions may lead us ahead in our mutual progress in improving the audiometric aspects of the neurophysiology of audition. There is, however a second point which has to be considered when talking about cortically evoked potentials. although we know for sure that the cor tically evoked response is due to both channels of information processing in hearing, it is not known whether each cortical response is the sum, or the difference of two types of information, namely (I) previously stored and (II) actually handled information.

Using prolonged sinusoidal tones as stimuli, Finkenzeller at our Department could show in cats with implanted electrodes that there is *some sort of electrical activity within the pe*riod between the on- and off-effect. This potential certainly does not contain the stimulus frequency in a synchronized time pattern, but its integral, mainly as a shift of the d.c.-component of the averaged cortical potential (Fig. 1) This observation encouraged us to look more carefully at the stimulus-locked d.c.-shift of the averaged cortical potential. We therefore changed our equipment to a complete d.c. sensitive amplifier device with a time constant of infinity and a control by a second identical d.c.-amplifier with a frequency range between 0 and a few hundred Hz. Electrodes were made from Ag-AgCl and implanted subdural above AI and AII in a cat. Some of the results are shown in Fig. 2 (David et al., 1969 *c*)

Similar results could be obtained in the meantime from the human scalp using Ag-

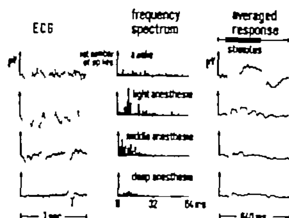


Fig 1 Only in deep anesthesia is the cortically evoked response to longlasting sinusoidal tone an on- and small off-effect (right row bottom figure) With decreasing depth of anesthesia (from bottom to top) the d c.-potential between the on- and off-effect develops and is clearly detectable in the wake state (right row top trace). Frequency spectrum of the ECG and EEG in the cat are recorded simultaneously (According to Finkenzeller unpublished data)

AgCl-sintered self adherent Beckman-electrodes with a diameter of about 3 mm located over the vertex and measured against the mastoid. Although it is not the easiest job to record

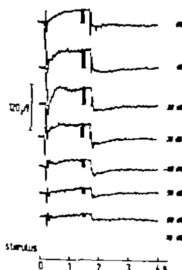


Fig 2 D C-potentials and classically evoked potentials (on- and off-effect) in the unanesthetized cat (chronically implanted electrodes). Auditory stimuli (prolonged sinusoidals). (David, Finkenzeller Kallert & Keidel 1969 c)

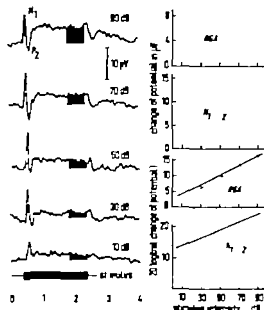


Fig 3 D C.-potentials and classically evoked potentials (on- and off-responses) in man to prolonged sinusoidal tones, averaging technique. Black areas and amplitude V_1–P are computed and plotted in a log-log-diagram revealing the steepness of the related intensity functions (four figures, right row). (David, Finkenzeller Kallert & Keidel, 1969 d)

d.c.-potential-shifts in man because of polarization artefacts, clearly reproducible results could be obtained, as shown in Fig. 3 Again, between the on- and off-evoked potential—using prolonged sinusoidal tones—a d.c.-shift during the tone can be recorded which is finished by the off-evoked potential This human d.c.-potential to prolonged tones can now be studied with amplifiers of a frequency range between real zero and a few hundred Hz. The potentials can be computed and their threshold and intensity functions can be measured, as shown in the right column of the figure. So for longlasting tones we certainly do not have just one evoked potential. Rather we have three parts of the compound potential, namely the initial evoked optential (the on-effect) the d.c.-shift during the tone and finally the evoked potential at the end of stimulus (the off-effect) All three parts of the potential can

be selected clearly All gradients of the intensity-functions differ and can be deter mined separately This again enables one to measure thresholds in different procedures by extrapolating the different intensity functions, which we did This is described in detail else where. Generally speaking, the three-part type (on-effect silent period periodic specific ac tivity) is the rule. This observation in turn allows one to compare the temporal pattern of colliculus-geniculate and cortical activity more in detail. Colliculus-cortexcomparison is shown first in Fig. 4

A temporal comparison shows clearly that the cortically evoked and averaged response

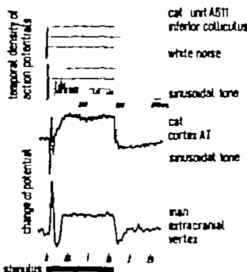


Fig 4 Comparison of the temporal pattern of colliculus and cortical activity to auditory stimulation i cat and man respectively The three upper traces are recorded in the cat, the bottom trace is an extra cranial record from the vertex in man. Duration of all stimuli is indicated by the cross hatched vertical strip and by the black line at the bottom of the figure. It can be seen that the on-effect in the PST-histograms before silent period precedes the intramodal specific auditory activation. This on-effect is some how related to the V potential, although this lasts during the silent period t colliculus-level and even a little bit longer The specific activation at colliculus is indicated by the fact that noise and sinusoidal tone are clearly represented in the envelope of the main part of the histogram. During this time in both the cat and in man the d.c.-potential can be observed which is cut off by the small evoked off-effect. (Own observations.)

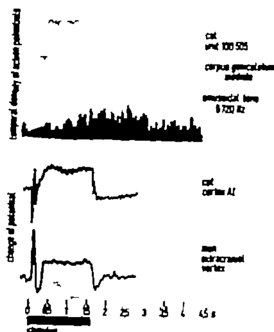


Fig 5 Same as Fig. 4 but here the PST-histogram is recorded from unit of the medial geniculate body (Own observation)

which we use for objective audiometry repre sents only the intramodal nonspecific, inter modal specific component of the response complex. Only the d.c.-shift, both in the cat and in man, occurs at that temporal period *when at levels lower than cortex the intra*modal specific information can be recorded. To make this statement more precise, in Fig. 5 absolutely identical time scales are used for geniculate and cortical responses. Again, the on-effect and the intramodal specific temporal period are very closely related and fairly similar in the pattern to the cortical evoked a.c. potential and to the average d.c. potential in cat and man respectively In the future, needless to say much more importance will be at-

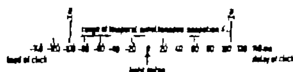


Fig 6 Range of temporal simultaneous sensation for a short sound and light pulse f medium stimulus intensity Standard deviation of the experimental results from Keidel, Breuing & Wiegand (1969).

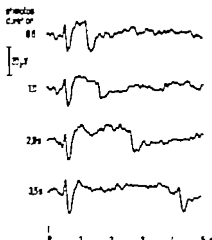


Fig 7 Stimulus-correlated d.c.-responses for different stimulus duration. Stimulus: Sinusoidal tone 1 kHz, intensity 70 dB, rr 1/10 sec. The various stimulus durations were applied at random. 300 tests were used for each curve. (David, Finkenzeller Kallert & Keidel 1969 d.)

taches to this potential in the course of the development of objective audiometry" than was the case in the past.

In conclusion, I would like to mention that the time delay from stimulus onset up to actual perception and information processing in the order of up to 200 msec and more seems rather long and not consistent with daily life experience. But, bear in mind that temporal division of time perception is of the same order (v

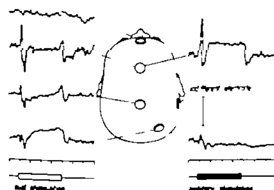


Fig 8 Vertex and occiput potential changes during application of light (left side) or tone (right side) The d c.-response for visual stimulation is greatest at the occiput, for auditory stimulation at the vertex (David, Finkenzeller Kallert & Keidel 1969 a, b.)



Fig 9 Vertex and occiput potential changes during application of sound (left side) and f sound combined with light (right side). T 50% of the tonal stimuli light was added at random. (David, Finkenzeller Kallert & Keidel 1969 a, b)

Baer 1962) (physiological moment) and that for instance a flash and a click cannot be separated temporally within a time period of the order of 200 msec. This was proved with modern equipment recently by Keidel et al. (1969) as shown in Fig. 6. This also explains why we perceive brief auditory stimuli like clicks or short tone bursts relative to the other senses as "now" when physically the stimulus onset has happened 200 msec earlier Measurements of v Békésy (1960) according to which full sensation level for a constant sinusoidal tone cannot be reached before 180 msec after the onset of the tone confirm this statement. That we do not become aware of this strange relation between physiological and physical time is due mainly to the incidence of an isomorphic representation of the outside world unconsciousness, and not to reality (see Hensel 1966 Reenpää, 1961 1966 Bergström, 1962 Keidel, 1962, 1964 Keidel et al 1969)

D C -potentials in animals were observed in different laboratories, for instance by Gumnit (1960 1961), Caspers (1960) but it was not possible to record them in man for many reasons which are described in detail elsewhere. But I would not like to conclude my brief review without saying just a few words on the possible consequences of the clinical use of this technique in objective audio-

must be taking place The DC component is unfortunately difficult technically and different DC responses add together without differences in latency to differentiate them. Does the topographical distribution of the DC component agree with the hypothesis that it is generated in the primary auditory (or visual) projection area?

J Groen. Mr Davis has shown that there is a clear similarity between On and Off-effect in cortical response. But in his last figure I believe to have observed that with slowly growing intensity there is an On-effect, but there is no Off-effect with slowly decreasing intensity So am I right to suppose that there is a difference between On- and Off-effect?

W D Keidel (Reply) to Mr *Davis*: Yes, there is a very clear maximum of amplitude for auditory evoked DC components over the vertex, and for visual evoked ones over the occiput.

to Mr *Groen.* The stimulus with slowly growing intensity starts with a very small amplitude and reaches a nonadapted ear whilst the stimulus with slowly decreasing intensity ends with a very small amplitude but reaches an adapted ear That might be a reason for the difference between the On- and Off effect. But nevertheless you can suppose that there are other reasons for the difference between On- and Off-effect.

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OBJECTIVE (E.R.A.) AND SUBJECTIVE (C.O.R.) AUDIOMETRY IN THE INFANT

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Abstract. We have measured the auditory threshold on 250, 500, 1 000, 2 000 and 4 000 Hz in 5 hearing impaired infants aged 1 to 4 years with Suzuki conditioned orientation reflex (C.O.R.) and the slow evoked potentials (E.R.A.) during Nembutal sleep. The threshold difference between the two techniques never exceeds ±30 dB and 75% of the cases are within the ±10 dB range. A statistical analysis shows that neither the age of the subject nor the frequency and intensity of the stimulus exert any influence on the results obtained.

The electric response audiometry (E.R.A.) is especially useful in young infants and babies as it can be very difficult or even impossible to make a reliable subjective audiogram. Everybody knows the importance of the early diagnosis and treatment of congenital hearing loss. The absence of subjective reaction on acoustic stimuli does not necessarily mean a peripheral hearing loss.

It is in precisely these babies and young infants that E.R.A. poses many difficult technical problems. When they are awake they hardly remain quiet sufficiently long and when they fall asleep not only does the E.E.G change but also the shape of the reaction obtained after repetitive acoustic stimulation. In Fig. 1 we compare the reaction obtained in an awake adult with the reaction of a sleeping child. The reaction is much larger and the latency much longer in the sleeping child than in the awake adult. Several authors have demonstrated that the depth of the sleep and not the age of the subject is the determinant factor: a deeper sleep gives an increase in intensity and in latency of the slow evoked potentials. Therefore, the reaction obtained is not only a function of stimulus intensity but also and perhaps much more of the E.E.G sleep stage of the subject. If we wish to do E.R.A. threshold measurements in sleeping children they have to remain in the same sleep stage for a sufficiently long time. There we have the very complicated problem of artificially induced sleep. The ideal product is not yet discovered—every author seems to use a different one. Personally we use Nembutal sleep and do our measurements in the high voltage slow (H.V.S.) sleep stage.

RESULTS AND DISCUSSION

We stimulate with pure tones of 250, 500, 1 000, 2 000 and 4 000 Hz of known I.S.O intensity. One test consists of 50 tone-pulses of 100 σ duration presented in both ear phones at random intervals from 1 sec to 3 sec. The earth electrode is placed on the left ear lobe, one differential electrode is placed 2 cm to the left of the vertex in an interaural plane and another on the right ear lobe.

These last 2 years we compared the hearing of 76 infants 1 to 4 years old with subjective and electric response audiometry. These infants were referred to us for the diagnosis or the confirmation of a suspected hearing loss. Eleven of these infants were completely deaf and

HVS Sleep

30.8 μV

1 sec

	500 Hz	1000 Hz	2000 Hz
Awake adult 90 dB			
Sleeping child 90 dB			

Fig 1 Comparison of the reaction obtained in an awake adult and in a sleeping child (high voltage slow sleep).

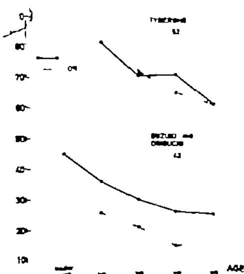


Fig 2 The measured hearing loss compared with Suzuki's and Origuchi's normal thresholds (Acta Oto-laryng. (Stockh.) Suppl. 252, p. 19 1969).

in 13 of them for some reason the subjective audiometry could not be performed (e.g. younger than 12 months, important mental deficiency or autism) So we have 52 children where we can compare the results of the subjective and the E.R.A. This comparative hearing examination was done in the following way: in the morning we test the hearing with Suzuki's conditioned orientation reflex (C O R.) technique and in the afternoon we use the same stimulation apparatus to do the E.R.A. during Nembutal sleep The only difference between the two techniques being free field for the C.O.R. and two ear phones for the E.R.A. Thus we could do with C.O.R. and E.R.A., 176 threshold determinations in 52 infants.

In Fig. 2 we compare the mean thresholds we obtained in the 4 examined age groups with the results published by Suzuki & Origuchi

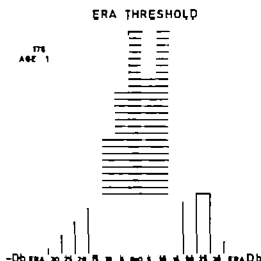


Fig. 3. E.R.A. versus C.O.R. thresholds taken as reference.

(1969) on 42 normal hearing infants examined with the same two techniques. Since our pathological curves are parallel to Suzuki's normal curves we may conclude that we found almost the same mean hearing loss in our 4 age groups.

The dispersion of the results has to be taken into account when we compare the C.O.R. and the E.R.A. thresholds. In Fig. 3 we have an almost symmetric dispersion that never exceeds ±30 dB. 75% of the cases being in the ±10 dB range. The mean difference is only 2.3 dB. A statistical analysis shows that neither the age of the subject nor the frequency and intensity of the stimulus exert any influence on the thresholds obtained.

CONCLUSION

We believe that under continuous E.E.G. control the E.R.A. gives reliable results in sleeping infants. However it remains a difficult and time consuming technique that should be especially useful in children and infants who can not be examined by subjective audiometry.

RESUME

Nous avons mesuré le seuil de perception auditive sur le 250, 500, 1 000, 2 000 et 4 000 Hz chez 52 enfants malentendants agés de 1 à 4 ans au moyen de deux techniques: d'une part le conditioned orientation reflex (C.O.R.) de Suzuki et d'autre part les potentiels évoqués cérébraux (E.R.A.) durant le sommeil provoqué au Nembutal. La différence des seuils obtenus ne dépasse jamais les ±30 dB et se trouve pour 75% des cas dans la zone de ±10 dB. L'age du sujet, l'intensité et la fréquence du stimulus employé n'exercent aucune influence sur la dispersion des résultats.

ZUSAMMENFASSUNG

Wir haben die Horschwelle auf 250, 500, 1 000, 2 000 und 4 000 Hz in 52 schwerhörigen Kindern (von 1 bis 4 Jahre) mit zwei Techniken gemessen: Suzuki' conditioned orientation reflex (C.O.R.) und die evozierte Potentiale (E.R.A.) während Nembutal Schlafes. Der Unterschied zwischen beide Schwellen ist niemals grösser als ±30 dB und 75% der Fälle sind in der ±10 dB Zone. Das Alter der Subjekte, die Intensität und die Frequenz des Stimulus beeinflüssen die Streuung der Ergebnisse nicht.

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DISCUSSION

H Davis: In St. Louis we are doing exactly the same type of study as Mr Tyberghein and we agree in all respects with the report that he has given. We are now engaged in an effort to measure objectively the depth of sleep, based on the amplitude of delta waves, in order to test only in the favorable deep stage. An approximate guide is the width of the EEG tracing on a very slowly moving paper It is necessary to wait for the favorable deep sleep, which may last, with proper dosage of seconal, for 30 or 40 minutes. This allows time for determination of thresholds at 3 frequencies (250 1000 and 3000 Hz) in each ear The reason for the difficulty is that we have two different responses, (1) the waking response in the stage of alpha activity and (2) the sleeping response, the K complex, which appears only in delta-wave sleep. Between the two is null stage when it is useless to test for responses.

Acta Otolaryng 71 253–261 1971

ELECTRO-COCHLEOGRAPHIE SUR LE NOURRISSON ET LE JEUNE ENFANT

Méthode d'audiométrie objective

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Abstract Ce test n'est pas seulement une méthode d'audiométrie objective mais surtout un examen fonctionnel de l'organe récepteur. Dès maintenant, son utilisation chez le très jeune enfant permet l'orientation précoce d'une rééducation la mieux adaptée. Le seuil, la forme de la réponse évoquée par le clic, ainsi que ses variations en fonction de l'intensité du clic (amplitude et latence) permettent de caractériser le fonctionnement de l'oreille testée et parfois de localiser les lésions éventuelles.

Le diagnostic de la surdité dans les premiers mois de la vie a évolué considérablement ces dernières années. Parmi les techniques objectives envisageables, l'électro-cochléographie paraissait la plus appropriée pour l'étude spécifique de phénomènes neuro-sensoriels périphériques (Portmann et al., 1967 Yoshie et al., 1967 Yoshie, 1968 Sohmer & Feinmesser 1967 Spreng & Keidel, 1967)

Contrairement à l'électro-encéphalographie, l'oreille périphérique n'est pas immature durant les premiers mois, n'a pas d'activité au repos, donc pas de bruit de fond gênant la lecture de la réponse. Enfin, les potentiels recueillis sont spécifiques, ils représentent une véritable scopie de ce qui se passe dans le nerf auditif lors de la stimulation. Par contre elle nécessite la mise en place d'une électrode.

Dans nos premiers essais, celle-ci est placée contre la fenêtre ronde, à travers une perforation ou une paracentèse (Portmann et al., 1968). Depuis lors, la méthode s'est considérablement simplifiée, l'électrode très pointue et rigide est directement piquée à travers le tympan sur le promontoire (Aran & Le Bert, 1968) La perforation punctiforme du tympan se referme instantanément après le retrait de l'aiguille. Une électrode de référence est placée sur le lobule de l'oreille.

Les potentiels recueillis par stimulation répétitive sont mémorisés et moyennés dans un calculateur Cette méthode a été utilisée chez l'enfant dès l'âge de quelques jours avec des résultats toujours fiables (Aran et al., 1969 Ruben et al., 1962)

Nous présentons ici l'étude de 103 oreilles toutes enregistrées sous anesthésie générale. L'âge s'étale de 21 jours à 8 ans avec un maximum de cas à 1 an. Tous les sujets plus vieux que 3 ans présentaient des troubles neurologiques ou psychiatriques interdisant toute autre technique audiométrique Quelques exemples de réponses particulières, chez l'adulte et l'enfant, sont également présentés.

MATERIEL ET TECHNIQUE

Les détails techniques ont déjà été exposés dans d'autres travaux (Aran et al., 1969) Rappelons les éléments principaux

— anesthésie générale par Kétamine
— stimulation par clic et sons brefs, clics filtrés à bande passante étroite (9 600 Hz, 4 800 2 400 1 200 600 et 300)
— réponses enregistrées sur bande magnétique avec une impulsion de synchronisation pour analyse ultérieure dans un moyenneur
— mesure de la réponse après élimination

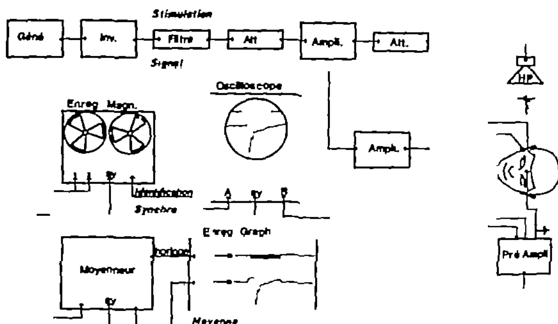


Fig 1 Matériel de stimulation et d'enregistrement.

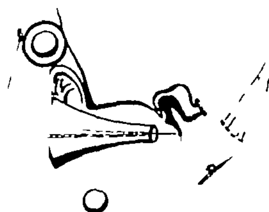


Fig 2 Position de l'électrode à travers le tympan.



Fig 3 Place de la piqûre transtympanique.

du microphonique, la réponse nerveuse est précisée dans son amplitude et sa latence

RESULTATS ET OBSERVATION

Les résultats sont remarquables du point de vue audiométrie clinique comme le montrent les quelques observations suivantes.

Sujets normaux ou légèrement atteints

No 203 Murielle H 8 mois

Histoire clinique les parents viennent consulter craignant une surdité l enfant ne paraît pas très intéressée par des bruits pourtant familiers.

A 3 mois, syndrome méningé avec hémiplégie gauche et paralysie faciale droite qui a régressé.

Tests audiométriques R.C.P et R.O C. positifs aux fortes intensités.

Jouets sonores réponses nettes aux moyennes intensités 60 dB (aigus), 80 dB (graves)

E.Co G alors que pour *l'oreille gauche* on trouve des réponses absolument normales avec

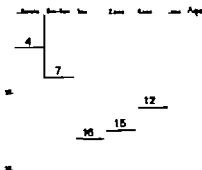


Fig 4 Répartition des enfants testés sous anesthésie générale et correspondant à la série de 103 oreilles sur lesquelles s'appuie ce travail. Tous les enfants au-dessus de 3 ans ne pouvaient être examinés par des méthodes audiométriques habituelles ou par électro-cochléographie sous anesthésie locale car ils présentaient des syndrômes neurologiques, psychologiques ou psychiatriques l'interdisant.

un seuil à moins 10 dB ce qui est extrêmement rare et une amplitude maximum importante (23 µV) il semble que, pour *l'oreille droite* malgré le seuil à 15 dB l oreille soit affectée, la réponse maximum n étant que de 2,2 µV ce qui est faible pour une oreille que l'on aurait pu considérer comme normale.

No 119 Oliver C., 5 mois

Histoire clinique agénésie de l'oreille gauche, absence de C.A.E.

Oreille droite anatomiquement normale qu'il parait nécessaire de vérifier par E.Co.G (l'oreille agénésiée ne peut être testée puisqu on ne peut y placer l'électrode). *Tests audiométriques* jouets sonores bien perçus (40–50 dB)

E Co G oreille droite réponses normales, seuil à 0 dB. D autre part, on a pu enregistrer les réponses aux différents sons brefs à 50 dB

No 188 Gérard T 6 ans et demi

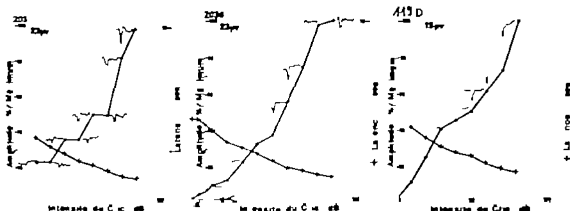
Histoire clinique accouchement normal, marche à 2 ans, possède alors dix mots de vocabulaire environ, puis régresse et actuellement ne dit absolument rien. Le comportement s est également détérioré et est devenu tout à fait autistique.

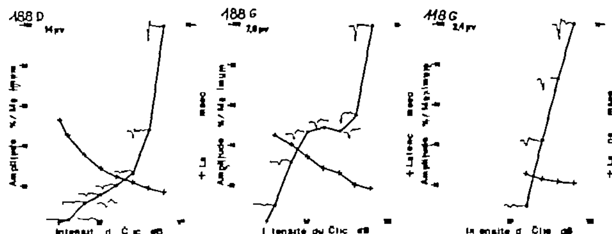
Tests audiométriques irréalisables.

E.Co G *oreille droite et oreille gauche* les seuils autour de 25 dB semblent normaux si l'on tient compte du fait que le bruit de respiration était important. Les formes de la courbe et de la réponse pour *l'oreille droite* sont cependant différentes de la normale sans que l on puisse, pour le moment, en tirer une conclusion. Eventuellement, cela pourrait rappeler certains types de surdité de perception associés à une symptomatologie neurologique plus complexe.

Surdités de réception avec recrutement

Il est remarquable de voir que le recrutement peut être objectivé de façon très précise. La comparaison avec les tests classiques (Balance, Luscher Shl, Békésy etc.) lorsqu elle





est possible, notamment chez l'adulte, nous a montré une concordance très appréciable. Trois symptômes sont caractéristiques, la rapidité de croissance de la courbe « input-output », la brièveté de la latence même au seuil, le caractère nettement diphasique de la réponse.

No 118 Anita V 2 ans

Histoire clinique gros retard psycho-moteur arriération probable Pas d'antécédent connu. *Tests audiométriques* peu précis. On obtient des réponses dispersées, réaction au nom à 100 dB réaction aux jouets sonores aux fortes intensités mais variable suivant que l'enfant est plus ou moins disponible. R.O.C. 100 dB

E Co G alors que pour *l'oreille droite* on n a obtenu qu un signal très faible à 100 dB qu'il est difficile d'interpréter comme une réponse on obtient, pour *l'oreille gauche* un seuil à 60 dB et des réponses très nettes au-dessus. On notera, au seuil, la latence relativement brève (2,5 ms) qui est très caractéristique.

No 113 Bernhard H 9 ans et demi

Histoire clinique surdité congénitale familiale ayant entraîné un important retard de langage. *Tests complémentaires* S.I.S.I. 100% pour les deux oreilles (1 000–2 000–4 000)

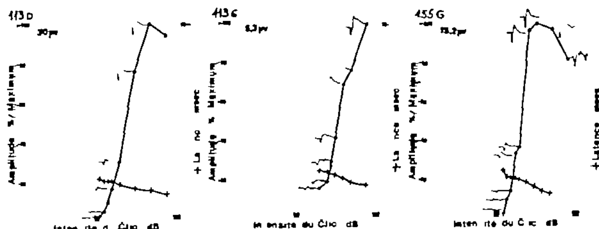
E Co G on trouve ici pour *les deux oreilles* les trois caractéristiques du « recrutement »

— latence brève au seuil
— augmentation rapide de la réponse
— forme diphasique.

No 155 Philippe E 9 ans et demi

Histoire clinique

— retard dans l établissement du langage



— surdité probablement congénitale aggravée par des épisodes otitiques et rhino-pharyngés actuellement guéris.

Tests complémentaires

— Weber latéralisé à droite.

— S.I.S.I. oreille gauche = 100 % (1 000-2 000-4 000).

E.Co.G oreille gauche exemple frappant de recrutement » non seulement la réponse augmente très vite pour atteindre une amplitude importante, mais elle décroît ensuite. Elle est en outre très diphasique.

On conçoit que cet enfant a, pour son oreille gauche, une *dynamique* très réduite. Le seuil, pour le 9 600 a été trouvé à 60 dB

Surdités avec formes électro-cochléographiques anormales

Dans quelques cas le pattern de la réponse est anormal. Il s agit toujours de sujets, présentant des surdités associées à des problèmes neurologiques.

A titre d'exemple, le cas no 104 D rencontré chez l'adulte est caractéristique il s agit d'une compression du nerf cochléaire par un neurinome du nerf facial dans le conduit auditif interne (contrôle opératoire des lésions).

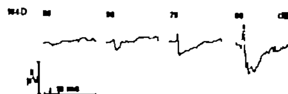
Nous retrouvons des patterns particuliers également chez l'enfant incompatibilité facto-maternelle no. 111 no. 165 infirme moteur cérébral no. 160 gros retard no 181 syndrome neurologique avec convulsion et aphonie no 148 etc.

Remarquons qu'il ne s agit pas d'artéfact dû à l'anesthésie générale, les mêmes formes se rencontrant sur l'enfant no. 148 en anesthésie générale et dans une deuxième séance, plusieurs mois après en anesthésie locale.

No 104 Monique J., 25 ans

Histoire clinique neurinome du nerf facial droit (compression du conduit auditif interne droit)

Tests complémentaires malgré le seuil à 75 dB à droite, la courbe vocale présente une forme en cloche très marquée (intelligibilité maximum 75 %)



E.Co G oreille droite réponses typiques constituées d'un pic positif précoce (potentiel récepteur sommation?) suivi d'une onde lente négative d'origine certainement nerveuse.

No 111 François L., 6 ans

Histoire clinique incompatibilité facto-maternelle par facteur Rhésus avec ictère nucléaire.

— incoordination motrice très importante

— instabilité

— gros retard de langage.

Tests audiométriques

— conditionnement impossible

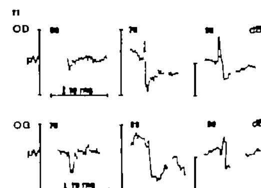
— réactions aux intensités moyennes (70 dB)

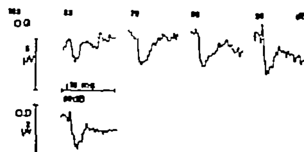
E.Co G cet enfant a bien entendu été testé sous anesthésie générale on trouve des seuils élevés en accord avec l'audiométrie et, *pour les deux oreilles* toujours cette même forme.

No 160 Fabienne G 6 ans 8 mois

Histoire clinique prématurité importante. L enfant est née à 6 mois. Léger ictère à la naissance.

Actuellement, infirme moteur cérébrale troubles moteurs importants de type athétosique la station assise est difficile, la marche impossible, malgré une rééducation motrice précoce.





Tests audiométriques malgré un conditionnement difficile en raison des mouvements athétosiques, un audiogramme bilatéral aérien est réalisé et laisse suspecter sans pouvoir l'affirmer une surdité à 70–80 dB

E.Co G *pour l'oreille droite et l'oreille gauche* on trouve toujours la même forme de réponse, le seuil *pour l'oreille gauche* entre 60 et 70 dB est meilleur que *pour l'oreille droite* (entre 80 et 90 dB)

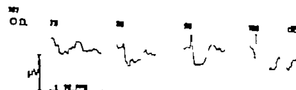
No 181 Eric T 2 ans et 8 mois

Historie clinique absence de langage, très gros retard moteur l'enfant s est assis à 18 mois et depuis quelques semaines réussit à se tenir debout en s aggripant à son parc. La préhension des objets ne dépasse guère le niveau de 7–8 mois.

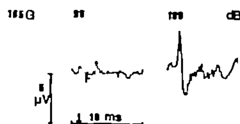
Dans les antécédents, on note

une incompatibilité faeto-maternelle par facteur Rhésus avec ictère nucléaire ayant nécessité trois exanguino-transfusions puis deux transfusions au cours du premier mois.

Tests audiométriques aucune réaction aux







jouets sonores. Aucune réaction au nom, même à 100 dB vague R.C.P au gong émis à intensité maximum. Le diagnostic de surdité profonde est envisagé mais le retard psychomoteur est trop accusé pour permettre de l'affirmer en toute sécurité.

E.Co G les réponses observées *pour les deux oreilles* présentent le même caractère que pour les autres cas. On notera cependant les seuils trouvés à 75 et 55 dB respectivement pour *les oreilles droite et gauche*

No 165 Raphaël G 9 ans

Histoire clinique enfant examiné pour la première fois à l âge de 7 ans pour absence de langage mise sur le compte de difficultés motrices (incoordination) jointes à un retard mental.

Incompatibilité faeto-maternelle par facteur Rhésus avec ictère nucléaire. Deux exanguino-transfusions.

Tests audiométriques après conditionnement facile, on objective une atteinte bilatérale sérieuse de type réception.

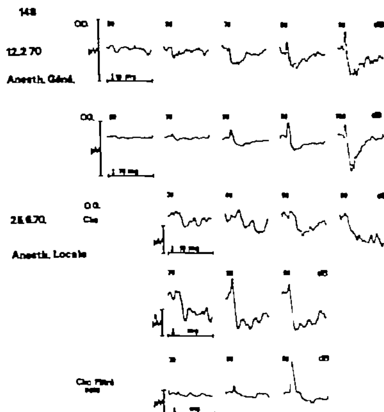
E.Co G sous anesthésie locale oreille gauche ici, bien que la réponse soit très faible avec un seuil entre 90 et 100 dB on retrouve la même forme anormale de réponse.

No 148 Jean-Marc V., 6 ans et demi

Histoire clinique grossesse et naissance normales.

A cinq mois et demi, petites crises convulsives avec, pendant deux mois, syndrome neurologique de West. L'E.E.G est actuellement normal.

Tests audiométriques malgré un audiogramme qui, tout au moins à gauche, pouvait laisser



espérer une rééducation facile et également un Q.I. normal, cet enfant, en centre de rééducation pendant plus d'un an, n a pu apprendre que 10 nouveaux mots environ.

E.Co.G cet enfant a *été* testé une première fois sous anesthésie générale. On a enregistré, *pour les deux oreilles* ces réponses anormales Devant le caractère particulier de ces réponses que nous observions depuis peu de temps, nous avons décidé de réaliser un nouvel examen sous anesthésie locale, afin de voir si ces formes particulières n étaient pas dues à l agent anesthésique utilisé ou si au contraire etaient reproductibles.

Ce test, réalisé 4 mois plus tard *pour l'oreille gauche* sous simple anesthésie locale, a *été*, malgré le jeune âge de l enfant, facilement réalisable. On a obtenu alors des réponses comparables et, malgré la faible amplitude de celles-ci, on a pu déterminer un seuil à 30 dB De plus, pour voir l'influence de la nature des stimulations sur la forme des réponses, nous avons également observé celles-ci lors des stimulations par clics filtrés à 9 600 Hz. Nous avons ainsi trouvé un seuil à 60 dB et un pic positif extrêmement important par rapport à l'onde lente négative d'une part, et par rapport aux réponses aux clics d'autre part. On se rappellera que dans quelques cas on a ainsi observé, à côté de réponses apparemment nor mal pour le clic, un pic positif similaire pour le 9 600

DISCUSSION

L électrocochléographie humaine est dès maintenant suffisamment au point pour être utilisée comme méthode d'audiométrie objective dès le plus jeune âge (Aran, 1970) à condition de se soumettre aux impératifs techniques que nous recommandons, notamment mise en place transtympanique de l'électrode

active directement sur le promontoire. Elle est appelée à un grand avenir pour l'étude physiologique et physiopathologique de l'oreille humaine (Aran, 1970).

Elle permet, chez le jeune enfant, de définir le niveau d'audition et même l'allure de la courbe audiométrique pour l'une et l'autre oreille. Ceci facilite l'utilisation de la prothèse auditive en éducation précoce dès l'âge de 5 à 8 mois.

L'étude de l'amplitude et de la latence des réponses en fonction de l'intensité du stimulus permet de définir les oreilles atteintes de recrutement. Cette analyse sera certainement fructueuse pour élucider les mécanismes pathogéniques de ce phénomène, si on le rapproche des travaux morphologiques des dernières années sur l'oreille (Lagourgue, 1970; Spoendlin, 1962; Spreng & Keidel, 1967).

L'apparition de formes anormales doit également ouvrir la porte à des recherches pathologiques (Aran, 1970). Elles correspondent toujours à des cas neurologiques particuliers. Leur analyse permettra peut-être un jour de trouver dans l'électrocochléogramme les signes différentiels nécessaires au diagnostic particulier de telle ou telle perturbation des mécanismes cochléaires.

SUMMARY

Threshold and pattern of the click-evoked peripheral nervous response as well as its variations (amplitude and latency) with the intensity of the click, characterize the working of the tested ear and can sometimes show up the localization of the disorder. This test is not only method of objective audiometry but mainly a functional examination of the peripheral receptor. Its use through the great variety of pathological cases should lead to new and precise diagnostics and to new ideas about the human hearing mechanism. Even now its use in very young children allows the early indication of the most suitable reeducational program.

ZUSAMMENFASSUNG

Bei diesem Test handelt es sich nicht nur um eine Methode objektiver Audiometrie sondern speziell um eine funktionelle Überprüfung des Empfangorgans. Bereits gestattet die Anwendung beim Kleinkind die frühzeitige Orientation einer Neuerlernung unter günstigen Bedingungen. Die Schwelle, die Form der durch den Klick hervorgerufenen Reaktion, sowie ihre Variationen im Verhältnis zur Intensität des Klicks (Anschwellen und Latenz) gestatten die Karakterisierung der Funktion des getesteten Ohres und in manchen Fällen das Aufspüren eventueller Verletzungen.

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DISCUSSION

H. S. Spoendlin. Mr Portmann showed that general anesthesia has no effect on the activity of the cochlear nerve. On the other hand central disturbances, such as incompatibility and psychological disorders appeared to have a distinct effect on the acoustic nerve activity. Do you have an explanation for this discrepancy?

H. Davis: Mr Portmann and Mr Aran have established electrocochleography as practical clinical and experimental procedure. It is now ready for refinement, particularly by the use of filtered clicks to separate the responses of the first turn from those of the upper turns. The action potential patterns agree well with predictions from animal experiments. The other features of summating potentials, afterpotentials, etc. should be greatly illuminated by the studies of Mr Groen and Mr Kupperman. Correlations with pathology may improve our understanding of these other potentials which contributed largely to the "abnormal" patterns shown by Mr Portmann. Ultimately the comparison of results from cochlear and cortical potentials respectively offer great and most interesting diagnostic possibilities.

R. Kupperman. (1) There is difference of latency found in the test animal and in these measurements performed in the human being. The question is whether this big latency is due to shift of the stimulus time due to shift of the peak power in the total stimulus. (2) The abnormal AP is often seen in hypoxic state of the cochlea. The question is whether these abnormal AP in the human being are also the result of cochlear and of neural metabolic disorders.

A. Montandon. Les très remarquables résultats présentés par le Mr Portmann soulèvent le problème d'une dissociation possible entre les lésions périphériques de l'appareil auditif et l'énorme complexe psychologique qui accompagne la perception auditive. Existe-t-il réellement des lésions objectivables qui correspondent au déficit auditif ainsi mis en évidence?

Peut-on, d'autre part, utiliser ce procédé pour permettre les indications d'un appareillage prothétique de l'enfant ?

M. Portmann (Reply) to Mr *Spoendlin:* Il n'y a pas contradiction entre absence d'influence du sommeil et perturbation par troubles centraux, car en fait les affections dites "troubles centraux" telles les lésions nerveuses dues à l'incompatibilité Rhésus sont aussi bien centrales que périphériques.

To Mr *Davis:* Je remercie Mr Davis pour son appréciation qui nous honore mon collaborateur J.-M. Aran et moi-même. Il est très important de retrouver chez l'homme normal les mêmes résultats que chez le chat ou le cobaye. Quant à l'homme pathologique, son étude aidera certainement à la compréhension des mécanismes physiologiques neuro-sensoriels cochléaires.

To Mr *Kupperman.* J n ai pas d'explication à donner pour comprendre la différence de latence constatée. J suis heureux de voir que Mr Kupperman trouve des formes anormales chez les animaux hypoxiques. Ce genre de rapprochement entre expérimentation animale et étude humaine sera très utile pour éclaircir la pathogénie de certains troubles auditifs neuro-sensoriels.

T Mr *Montandon.* En effet on ne trouve pas toujours de "lésions" en fait, les troubles sont souvent infra-lésionnels, il faudrait s adresser si cela était possible, à l'électromicroscopie ou même à la cytochimie pour trouver les déviations fonctionnelles expliquant la surdité! Quant à l'intérêt de l'E.Co.G pour l'appareillage précoce, il est essentiel.

Madame Cl. Portmann occupe au Laboratoire d'Audiologie Clinique de Bordeaux de diriger une équipe de pré-rééducation précoce. Dès l'âge de 5 à 8 mois, les enfants y sont appareillés. L'E.Co.G. lui apporte des précisions (niveau de chaque côté, distorsion supra-liminaire, etc.) essentielles pour adapter au mieux l'amplification de chaque enfant et de chaque oreille.

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EFFECT OF TEMPORAL SUMMATION ON THE HUMAN STAPEDIUS REFLEX

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Abstract The sensitivity of the acoustic stapedius reflex as a function of stimulus duration was studied by monitoring acoustic-impedance changes at the ear drum. Our results indicate an unexpectedly pronounced duration effect. The sound intensity required for a criterion response had to be lowered by about 25 dB when the signal duration was increased from 10 to 100 msec.

Numerous experiments have established that the loudness of short sounds grows when their duration is increased. The phenomenon can be explained in terms of temporal summation. It is found that the loudness level increases by roughly 10 dB when the duration of the stimulus is increased by a factor of 10. This relationship is established for sounds with narrow frequency spectra at all practical intensity lev including the threshold. Broad-band noise at threshold level constitutes an exception. Under many circumstances, the auditory system acts as if it were a linear energy integrator One well-known anomaly as compared to truly linear integrators, shows up in the time con stant that decreases from about 200 msec at near threshold levels to 100 msec or less at moderate and high levels.

The exact locus of the auditory temporal summation is unknown. Nevertheless, several experiments have indicated that it is in the central nervous system (Zwislocki, 1960 1969)

It is well known that acoustic stimulation of high intensity (80-90 dB S.L.) is followed by a bilateral change in the acoustic impedance of the ear These impedance changes are brought about by reflex contraction of the stapedius muscle (Metz, 1946 Jepsen, 1955 Klockhoff, 1961 Salomon & Starr 1963 Møller 1962, Djupesland, 1965 1967) According to available experimental evidence, reflex contractions of the stapedius muscle are produced by neural activity in the superior olivary complex. Therefore, the contractions should reflect the temporal auditory summation only if the summation occurs at or below the level of the superior olive Some temporal summation occurs in the muscle itself, but its time constant is on the order of 50 msec rather than 100 or 200 msec (Galambos, 1956 Zwislocki, 1960) The effect of temporal summation on the acoustic stapedius reflex may be determined by measuring the reflex threshold as a function of stimulus duration. We undertook such measurements together with some suprathreshold ones.

MATERIAL AND METHODS

Six subjects, 4 female and 2 male, took part in the experiments. Their age ranged from 19-23 years. The selection criteria were: no observable pathology upon otoscopic examination, and normal hearing.

The reflex changes in the acoustic impedance at the eardrum were detected with the help of the Zwislocki acoustic bridge (Grason-Stadler Co., type E 8872A) The output of the bridge was picked up by a small microphone, amplified, filtered, and fed to a voltmeter as well as to an oscilloscope. In the main experimental series, the amplification, filtering and

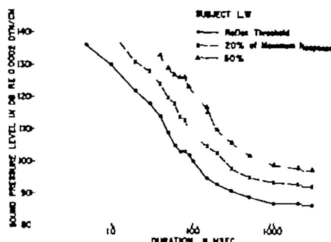


Fig. 1. Relationship between sound intensity and stimulus duration at three constant response levels of stapedius muscle. Individual data.

voltage indication were performed by a GR Wave Analyzer 1900-A, and the filter band width amounted to 50 Hz. The obtained results were checked by means of another electronic set-up with a reduced noise level and a filter bandwidth of about 500 Hz. The long time constant of 20 msec inherent in the first set up was the main reason for checking. The muscle time constants were found to be appreciably longer than 20 msec and the results of both series were essentially identical. The bridge signal consisted of a continuous 500 Hz tone maintained at a comfortable sound intensity well below the reflex threshold. The muscle reflex was elicited contralaterally by means of 2 000 Hz tone bursts of variable duration and intensity. The rise and fall times of the bursts amounted to 5 msec and the duration was measured between the half-power points. It was varied within the range 5–3 000 msec. The bursts were repeated at a rate of one per 5 sec to avoid any noticeable reflex interactions. The burst intensity was controlled by means of a Hewlett Packard attenuator set (350 BR) in 1 dB steps.

The experiments began on every subject with the measurement of the ear-canal volume whose effect was subsequently eliminated by an appropriate bridge setting. As a next step, the bridge was introduced into the ear canal and was balanced. The absolute impedance at the eardrum was noted. Next, the bridge was slightly unbalanced in order to produce a near maximum sensitivity to impedance changes. The latter were indicated by the vertical amplitude on the oscilloscope tube. The stimulus intensity was adjusted so as to satisfy one of the following three criteria, minimum visual detection level (VDL), 20% and 50% of maximum response.

RESULTS

The results are shown in Figs. 1 to 5. The first figure shows the relationship between the stimulus duration and the sound intensity required for eliciting a just visible impedance change, 20% and 50% of maximum response. The points indicate the means of three measurements carried out on the same subject. The duration of the tone bursts in milliseconds is plotted on the Y-axis. The sound pressure level in dB re 0.0002 dyn/cm² is plotted on the Y-axis.

The results indicate that the duration of the tone bursts has a pronounced effect on the stapedius response. The effect is about the same for all criterion levels. For this subject, the sound intensity had to be lowered by about 30 dB when the signal duration was increased from 10 to 1 000 msec in order to maintain the criterion responses.

The shift of the reflex threshold obtained on a group of 6 subjects as a function of stimulus duration is shown in Fig. 2. The

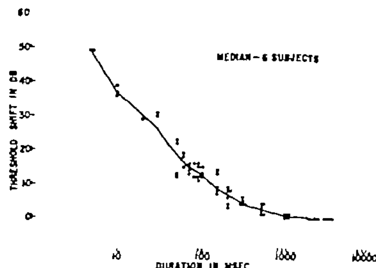


Fig 2. Relationship between sound intensity and stimulus duration at threshold of stapedius reflex. Circles indicate individual means, curve goes through medians.

individual results are plotted by means of dots. The curve is drawn through the population medians. As is indicated by the curve, the median threshold intensity had to be lowered by about 25 dB when the duration of the tone bursts was increased from 10 to 1 000 msec. This is about 2.5 times as much as would be required for constant loudness.

The results indicate that the effective time constant of integration is about 200 msec—the same as for the threshold of audibility

The following three figures refer to the ustic bridge output as seen on the oscillo-pe screen. The output voltage is roughly proportional to the impedance change It is indicated by the width of the upper trace. The lower trace indicates the stimulus duration. In Fig. 3 is shown the effect of a 500-msec tone burst. The time scale is 100 msec per division. Note the slow rise of the response, indicating a long process of temporal summation. The decay appears to be somewhat faster but this is mainly due to an optical illusion. A closer examination indicates similar rise and decay time constants. A just visible impedance change elicited by a 10 msec tone burst is shown in Fig. 4 The time scale is again 100 msec per division. Without changing the sound intensity the duration of the stimulus was increased from 10 to 100 msec. Fig 5 shows the resulting response It is much larger than for the shorter burst.



Fig 3 Bridge output voltage (upper trace) as seen on oscilloscope screen during stapedius contraction, and voltage proportional to stimulus sound pressure. Time scale: 100 msec/division. Stimulus duration: 500 msec.



Fig 4 Same as Fig. 3 for near threshold response *Stimulus duration: 10 msec.*

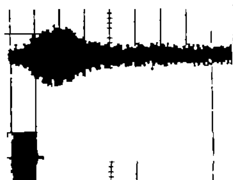


Fig. 5 Same as Fig. 3 for ten times longer stimulus.

DISCUSSION

The results illustrated in the figures clearly indicate that a temporal summation with a time constant on the order of 200 msec affects the acoustic responses of the stapedius muscle. Since the temporal summation that takes place in the muscle itself has a considerably shorter time constant (about 50 msec), we assume that the long-term temporal summation is the same as for the threshold of audibility and loudness. If this assumption is correct, the site of the temporal summation must be located within the reflex arc of the stapedius muscle. This arc does not seem to extend beyond the superior olive. As a consequence, the temporal summation should take place in the superior olive, or below. A lower location is unlikely since the long-time constant would make the auditory system insensitive to small dichotic time differences that are required for precise localization of sound. Occasionally long response latencies are found in single units of the cochlear nucleus, but these latencies may be due to the activity of the efferent system. Therefore the superior olive emerges as a likely site of the psychophysically apparent temporal summation.

RÉSUMÉ

La sensibilité du réflexe acoustique de l'oreille moyenne en fonction de la durée du stimulus a été étudiée en observant les changements de l'impédance acoustique au tympan. Nos résultats indiquent un effet de durée plus prononcée que nous avons attendu. L'intensité du son qui était nécessaire pour obtenir une réponse satisfaisant à notre critérium a dû être diminuée d'environ 25 dB lorsqu'on augmenta la durée du signal de 10 à 100 msec.

ZUSAMMENFASSUNG

Der Einfluss von der Reizdauer auf den akustischen Stapedius Reflex beim Menschen wurde durch Änderungen der akustischen Impedanz des Ohres untersucht. Unsere Messungen zeigen einen unerwartet grossen Einfluss von der Dauer des Tonreizes. Um dieselbe Änderung in der akustischen Impedanz hervorzurufen, musste die Intensität des Tones um ungefähr 25 dB reduziert werden wenn die Dauer von 10 zu 100 ms. verlängert wurde.

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DISCUSSION

H. Davis: There are several ways to measure a time constant of the auditory system. The stapedius reflex is one of them. The behavioral threshold is another and supra threshold loudness balance is another. These three all give large values, 100 to 200 msec periods of summation. It is wrong to think, however of a single time constant of the auditory system. There are probably several of them. For example for the slow vertex potential there is no further increase in amplitude if a tone-burst is prolonged beyond 30 msec. This is clearly shorter than 100 msec.

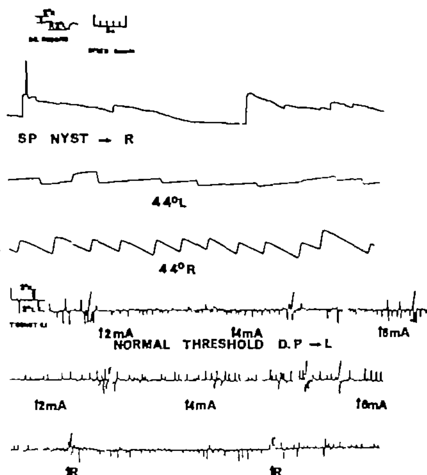


Fig 1

mation from the remaining intact labyrinth. This is realized on the one side by the neuronal connections between the labyrinth and the vestibular nuclei, on the other side by the feed back circuits connecting the primary vestibular centres bilaterally with the superimposed integrational centres of the reticular formation. It is well known that the reticular information is capable of modifying the reception and integration of sensory signals at the first synaptic level (vestibular nuclei) to the degree that some will be perceived and others rejected (Gernandt, 1967) On the other hand the afferent flow of impulses in many sensory systems is modified by activity in specific efferent fibres. According to Sala (1965) these findings also hold true for the vestibular efferents originating from the vestibular nuclei and the reticular formation as known from neuro-anatomical studies in cats and rabbits (Gernandt, 1967).

This feedback mechanism working upon vestibular responses may also be studied in nystagmograms recorded in patients suffering a sudden or progressive loss of vestibular function. Modification of spontaneous nystagmus on the one side and modification of the galvanic responses on the other side give us some information about this specific *accommodation mechanism* of the vestibular system.

Depolarization is equivalent to an increase *of sensory cell activity and action potential frequency within the first neuron, hyperpolariza-*

H.B. ♂ 1944 (135/69) (2)
3 MONTHS LATER

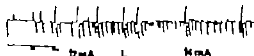
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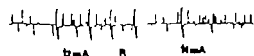
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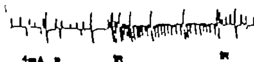
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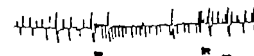
↑R ↑R ↑R
NORMAL THRESHOLD
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(INHIBITION OF NYST → R
– CENTRAL COMPENSATION)

Z.J. ♀ 1901 (152/69) (3)
POSTOPERATIVE FINDINGS
10 DAYS AFTER LABYRINTHECTOMY (L)









NORMAL THRESHOLD
INHIBITION OF NYSTAGMUS → R
(CENTRAL COMPENSATION)

Fig. 3

Fig.

tion is causing the opposite effect (Sala 1965, Trincker 1965). This is exactly the mode of action of galvanic stimulation which we have been using for this particular study. *Cathodal stimulation* corresponds to depolarization and produces a distinct increase in the frequency of the resting potentials, whereas anodal *galvanic stimulation* (corresponding to hyperpolarization) reduces the resting potentials to the point of total inhibition (Sala 1965).

In a *partial lesion of the vestibular end organ* the first symptoms of asymmetry may already be demonstrated within a very short time (1–2 days after the onset of the dysfunction) by the galvanic test. Unilateral stimulation reveals symmetrical thresholds in spite of the presence of a spontaneous nystagmus towards the normal side but the frequency of the nystagmus directed towards the side of the lesion is already increased. [illegible] tions produce a directional [illegible] wards the side of the lesion which [illegible]

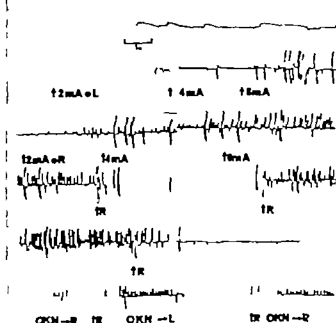



Fig 4

inhibition of the nystagmus directed towards the normal labyrinth, by decreasing its amplitude but not its frequency (Figs. 1 2)

After a *sudden and complete destruction of the vestibular endorgan* spontaneous nystagmus towards the unimpaired labyrinth may be observed for several weeks, reducing gradually its intensity with progressing accommodation. In nystagmograms recorded already 6 days after labyrinthectomy we may find suppression of the spontaneous nystagmus towards the normal side by galvanic stimulation and symmetrical threshold values. Supraliminar stimulation, however still reveals the presence of a directional preponderance corresponding to the *spontaneous nystagmus*. Four days later accommodation is completed. Spontaneous nystagmus has disappeared already at threshold level there is a marked directional preponderance towards the side of the lesion. This is due to an *inhibition* of the galvanic nystagmus directed towards the intact labyrinth (decrease of frequency and amplitude) and to a facilitation of the galvanic nystagmus directed towards the side of the lesion (increase of frequency and amplitude Fig. 3)

A longstanding total functional loss with complete accommodation may still show a spontaneous nystagmus towards the intact labyrinth, yet galvanic stimulation reveals perfect symmetrical reactions at and above threshold level, but on both sides supraliminar responses are considerably reduced by inhibition (decrease of amplitude)

Unilateral complete or partial lesions of the vestibular nerve i.e. a neuronal lesion, do not show analogous modifications of the vestibular responses by means of inhibition and facilitation of spontaneous and galvanic nystagmus (Fig. 4).

Differences in the procedure of "*central compensation*" substituting peripheral vestibular deficiency by optic and somatosensory regulations and by accommodation may now be explained on the one hand by disorders of the central nervous system, causing disturbances at the level of reticula formation and of the vestibular nuclei. We have to keep in mind that those structures represent a "station for programming and distributing vestibular signals through higher order neurons, constituting a link of utmost importance in amalgamating and adjusting activities so that the body is maintained at the best possible position to respond to the necessities imposed by the often changing environment" (Gernandt, 1967). On the other hand the integrity of the efferent vestibular system must be regarded as an equally important causal factor because it is the morphological and physiological basis of vestibular accommodations, modifying the response from the remaining intact labyrinth by adjusting them to a unilateral functional deficiency. As long as the peripheral vestibular neuron remains intact on the side of the peripheral lesion (endorgan) *accommodation* will be completed in a very short time. Because in man secondary degeneration of the first vestibular neuron following destruction of one labyrinth cannot be confirmed (Pfaltz, 1969) we may assume that modulation of the activity of the unimpaired vestibular endorgan by means of the efferent vestibular system is highly influenced by the activity of the still functioning vestibular ganglion cells on the side of the lesion.

RESUMÉ

Dans un groupe de malades, atteints d'une lésion vestibulaire périphérique strictement unilatérale, les auteurs ont essayé de faire l'étude de l'évolution de la compensation centrale, en moyennant des examens vestibulaires répétés sous contrôle nystagmographique. La compensation centrale des troubles vestibulaires dus à une fonction périphérique déficitaire se base sur deux mécanismes différents:

1. Un phénomène vestibulaire spécifique qui dépend uniquement de l'intégrité du système vestibulaire efférent. Il s'agit d'un processus d'accommodation c'est-à-dire d'une modulation des réponses du labyrinthe intact par moyen des fibres efférentes soit par inhibition soit par facilitation. Ce processus d'accommodation est généralement complété dans un délai très court. Une lésion au niveau du ganglion et du neurone périphérique engendra une accommodation incomplète et retardée.

2. Le second mécanisme est un phénomène central et non spécifique. Il s'agit d'une substitution des fonctions vestibulaires périphériques déficitaires par moyen de régulations optiques et somatosensorielles. Il en ressort que le terme de *compensation centrale* doit être réservé uniquement pour ce dernier mécanisme qui dépend de l'intégrité fonctionelle du système nerveux central. Une lésion cérébrale d'origine traumatique ou vasculaire empêchera l'achèvement d'une compensation centrale selon la sévérité des troubles lésionnaires cérébraux.

ZUSAMMENFASSUNG

Die vorliegende Arbeit beruht auf den Ergebnissen wiederholter otoneurologischer Untersuchungen, die bei Patienten mit einseitigem peripherem vestibulärem Funktionsausfall unter nystagmographischer Kontrolle durchgeführt worden sind.

Die zentrale Kompensation peripherer [illegible] Funktionsausfälle beruht auf zwei völlig verschiedenen Mechanismen. Im einen Fall handelt es sich um einen spezifisch vestibulären Vorgang, der [illegible] Anpassung, Angleichung oder *Akkommodation* bezeichnet werden kann und weitgehend vom efferenten vestibulären System bestimmt wird. Mittels Inhibition und Fazilitation, d.h. Hemmung und Bahnung werden [illegible] Reizantworten der verbleibenden intakten [illegible] moduliert und so dem Umfang der Funktionseinbuße auf der Gegenseite angepasst. Die Vollendung solchen Akkommodationsprozesses vollzieht sich in sehr kurzer Zeit, hängt jedoch von der Integrität [illegible] Ganglion- und Faserelemente des peripheren Neurons ab. Eine Schädigung derselben führt zu einer unvollständigen oder zumindest verzögerten funktionellen Angleichung.

Im andern Fall handelt es sich um einen unspezifischen Funktionsvorgang, der sich innerhalb des Zentralnervensystems abspielt: optisch-visuelle sowie somatosensorische Regulationsmechanismen kompensieren den spezifisch vestibulären Funktionsausfall. Der Begriff der "zentralen Kompensation" sollte deshalb diesem Mechanismus vorbehalten bleiben. Er ist abhängig vom Funktionszustand des *Zentralnervensystems* und wird deshalb durch traumatisch oder vaskulär bedingte Gehirnschädigungen wesentlich eingeschränkt.

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Acta Otolaryng 71 273–277 1971

COMPARATIVE STUDY OF THE ROTATORY VESTIBULAR NYSTAGMUS THRESHOLDS OBTAINED BY MEANS OF CONSTANT OR SINUSOIDAL ANGULAR ACCELERATION

Preliminary Report

A. Montandon S Huguenin, W Lehmann and F John

From the E.N.T Clinic University of Geneva Genève Switzerland

Abstract. The new vestibulometry using nystagmography requires an accurate valuation of ENG traces. This procedure as well as the statistical analysis are today remarkably well facilitated by the computer By these means we *have tried to compare two diffe*rent tests allowing us to quantify the rotatory stimulus in order to find possible correlation between the nystagmus thresholds.

A *frequency threshold* as was pointed out recently at the ENG Symposium of Geneva (1969) may be considered at several levels of stimulation, i.e., (*a*) *level of emergence* of signal (first beat) which can be observed directly on the traces (ascending threshold) (*b*) *zero-level* where the signal disappears (descending threshold) (*c*) *maximum frequency level* (1 Hz) corresponding to a given stimulus (*threshold of reference*)

METHOD

24 persons were tested by both methods, 17 normal and 7 pathologic.

Stimulus

(1) *Constant angular acceleration* (CAA): Chrograph of high precision, 5 h.p electric motor with hydraulic variator 3 clockwise and 3 counterclockwise angular accelerations at 0.8 /s^2 1 /s and 2 /s^2 followed by a sudden stop at 90 /s and an acceleration at 3 /s^2 followed by a slow deceleration at 4 /s which gives a total of 10 stimulations.

(2) *Sinusoidal angular acceleration* (SAA): Oscillating system. 20 periods of 20 sec, max. amplitude 180 max. acceleration of about 18 /s (*the exact value is calculated each time* by the computer)

Recording

Both examinations are made with open eyes in complete darkness. ENG in horizontal and vertical derivation, d.c. and a.c. (0.3 s) amplification. Separate slow recording of the movements of the frame at a speed of 2 mm/s.

Analysis of the traces

CAA Correction of acceleration and determination of average values of frequency at the maximum steady levels. Calculation by means *of a computer (CDC 3800)* *accelerations in* log. slope of regression line, valuation of errors, calculated thresholds at 0 level and at 1 Hertz in logarithms and in real values.

SAA Counting of the number of beats by half period (10 sec) and by side (right, left) measurement of the amplitude of each corresponding oscillation period of the seat. By means of a computer calculation of the max. acceleration of each period logarithmic transformation of the acceleration values, calculation of the slope and intercept of regression line.

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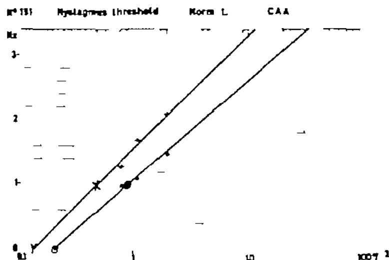


Fig. 2. Nystagmus thresholds by constant angular acceleration CAA (normal case).

zero level, 0.7 /s² (0.5–1.2) and 10 /s (0.5–49) at 1 Hz.

It must be emphasized that a similar logarithmic correlation between the stimulus of constant angular acceleration and the nystagmic response has been found, as was formerly demonstrated for the sinusoidal stimulus.

For both procedures *the nystagmus frequency varies as the logarithm of the stimulus* (Fig. 2).

Two further remarks must be made concerning the results. First, the sinusoidal nystagmic thresholds are systematically higher or even much higher than the unidirectional ones, due to constant angular acceleration (Figs. 2, 3) in the same individuals. That difference rapidly increases above the nystagmus frequency of 1 Hz as has been shown considering the stimuli that are needed to reach 2 Hz. Secondly the limits of variation at each level are evidently larger for the sinusoidal thresholds.

Some cases show a good concordance between the two methods, others do not. But hitherto we could find no satisfactory explanation for such disagreement.

Pathology showed similar concordant and controvertible results, especially when asymmetry of thresholds or latent nystagmus are concerned. Further research is therefore necessary before drawing conclusions.

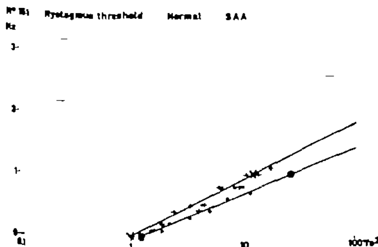


Fig. 3. Nystagmus thresholds by sinusoidal angular acceleration SAA (normal case).

COMMENT

The logarithmic correlation between angular acceleration, whether sinusoidal or constant, and nystagmus needs to be pointed out, contrary to the central electric stimulus, which is linear It suggests the possible existence of a particular property of the vestibular receptors and a similar mode of stimulation in both procedures.

At a low rate of stimulus, near the emergence thresholds, there is no large difference between CAA and SAA. The divergence becomes progressively more important on reaching higher frequencies of nystagmus, at 1 Hz and above. This requires a relatively higher efficiency of the sinusoidal stimulation at a low level of intensity than at a stronger one. The same divergence appears concerning the extent of individual variations in the value of frequencies at different levels of stimulation. Consequently we may say that despite of some concordance, no linear correlation was observed between the two methods.

In practice, in order to avoid any confusion, it should be remembered that torsion swing and continuous acceleratory rotation are not equivalent, but complementary tests. Both give luable though different information concerning the function of the vestibular system and eir quantification. It is therefore necessary to specify whether the nystagmus frequency threshold is set at zero, at emergence or at 1 Hz and which method of testing is used.

RÉSUMÉ

Le seuil d'émergence ou de référence du nystagmus obtenu chez les mêmes sujets par l'utilisation de deux méthodes différentes de stimulation d'accélération angulaire, sinusoïdale ou constante permet de comparer les valeurs respectives de leur paramètre fréquentiel.

ZUSAMMENFASSUNG

Die Drehbeschleunigungsschwelle für Nystagmus bestimmt durch die Erscheinung des Nystagmus oder durch eine gewisse Nystagmusfrequenz ist mittels zwei verschiedener Reizmethoden, sinusoidalen oder konstanten Beschleunigungen studiert. Dies erlaubt ein Vergleich zwischen ihren respektiven Frequenzparametern.

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DISCUSSION

M Portmann. La question du seuil est très complexe. Le zéro calculé" des courbes montrées par Mr Montandon me paraît très artificiel et donc de peu de valeur clinique. Ce n est que l'extrapolation des courbes des réponses obtenues dans une autre zone. Il est possible que la forme de la courbe soit autre chose que la droite sur laquelle le seuil calculé" est basé?

J J Groen. I gree with Mr Montandon upon many things, but we disagree upon one, which is the choice of the unit of stimulation. You use the angular acceleration and thus you will find a discrepancy in one test subject between his responses (expressed in angular acceleration) to constant and sinusoidal stimulation When the correct stimulus unit is used, which is the angular velocity this discrepancy will disappear

J Tonndorf Continuing along the lines of Mr Groen's comment, I would like to say the following: Mr Montandon showed us that, with sinusoidal stimulation, frequency is related to *log* acceleration. In my work this means that it is actually related to the integral of acceleration, e., to velocity

L. B W Jongkees: I have three remarks: (1) I am of the same opinion as Groen and Tonndorf that the parameter for stimulation is acceleration times and not acceleration. (2) Mr Montandon found dif ference between left and right. In what sequence did you give the stimulation in these experiments? Adaptation may play a paramount part. (3) How long was the duration of the continuous rotatory acceleration? After about one minute of acceleration the nystagmus has disappeared in normal people by adaptation.

A. Montandon (Reply) to Mr *Portmann*. La droite de régression calculée, par laquelle nous avons pu faire une comparaison au niveau O et au niveau de 1 Hz, est établie sur le base de plusieurs stimulations à différents niveaux d'intensité et son existence est prouvée par l'analyse statistique. Elle correspond à une méthode classiquement utilisée dans l'épreuve pendulaire.

L'absence de seuil auquel il a été fait allusion dans une accélération sinusoïdale, alors qu'il existait des seuils nystagmiques normaux à l'épreuve d'accélération angulaire constante démontre simplement l'absence d'une corrélation quantitative entre les deux méthodes dans ce cas particulier.

To *Mrs Groen and Tonndorf*: The constant rotatory stimulus we have used is of course an angular acceleration as well as the sinusoidal acceleration. But it must be recalled that the object of this study was to establish what degree of correlation can be found between two vestibular tests which are frequently considered as equivalent in clinical work.

To Mr *Jongkees*. Using a constant angular acceleration and not sudden stop the duration of the vestibular nystagmic response may last at least 3 minutes without ceasing or changing (1 /s^2 during 180).

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MORPHOLOGICAL AND HISTOLOGICAL CHANGES RESPONSIBLE FOR THE DROOP OF THE NASAL TIP IN ADVANCED AGE

J Krmpotić Nemanić, I. Kostović, P Rudan and G Nemanić

From the Anatomy Department Drago Perović" of the Medical Faculty and from the Otolaryngological Clinic of the General Hospital D Mladen Stojanović" in Zagreb Yugoslavia

Abstract The droop of the nasal tip in advanced age is due to the changes of the shape and of the structure of the lobular cartilage. In young people this cartilage is convex and rather resistant. In advanced age it is straightened and segmented so that it becomes larger in cranio-caudal direction and it thus shifts caudally

In advanced age a drooping of the nasal tip can be observed. This fact has been described in Belgium by Hoyer (1925) and in France by Bertillon (1925) However no explication has been given.

In order to explain this fact we have performed investigations in persons of different age groups, in cadavers and on histological sections through the nasal cartilages.

To be able to judge better the changes of the structures of the external nose in advanced age we have divided the external nose in three parts, i.e. the fixed part composed of the nasal bones, the semimobile part formed by the upper lateral cartilage and the mobile part formed by alar or lobular cartilage. The term alar cartilage is not suitable since the ala of the nose does not contain any cartilage because it is formed by two layers of skin. The inferior margin of the lobular cartilage corresponds to the alar sulcus.

In the persons of different age groups we have found as follows:

1 In children the relation between the three parts of the nose is 0 1 1 which means that the osseous part is not yet developed. The nasolabial angle is, in general, greater than 90 The nasal tip is firm and does not change its position by inclination of the head towards the left or the right.

2. Between 14 and 21 years the relation between the three parts is 1 1 1 The nasolabial angle is 90

3 In persons between 35 and 56 years there can be noticed a drooping of the nasal tip and its inclination towards the left or the right side on inclination of the head. It is more pronounced in females.

The drooping of the nasal tip is still more pronounced between 68 and 96 years of age. In these persons the semimobile and mobile parts of the nose are much longer than the fixed part. The nasolabial angle is affected by the presence of teeth or the wearing of a prosthesis. In both cases the naso-labial angle is under 90 If the person has no teeth and does not wear a prosthesis a paradoxal increase of the nasolabial angle can be observed due to the reduction of the alveolar process (Fig. 1)

By dissecting the external nose in 20 bodies in the age range 1–90 years we have found that in children and in young adults the alar cartilage is convex laterally Its upper border is curved inwards, forming one or two rectangles. The cartilage is situated in such a way that it covers the inferior part of the triangular cartilage. The latter is also curved towards the lateral side as has been already described by



Fig. 1 The nasal cartilages and their fragmentation in old persons.



Fig. 2 Left: the upper lateral and the lobular cartilage of young persons is represented. *Right:* there can be seen a fragmentation in old people.

Pech & Cannoni (1969) In advanced age the lobular cartilage becomes thinner and looses its convexity Its superior border is stretched and shows a fragmentation resulting in separation of small pieces of cartilage. These pieces form the accessory cartilages. At the same time a fragmentation of the inferior border of the triangular cartilage takes place.

The connective tissue between the upper lateral and lobular cartilages becomes less solid resulting in a larger distance between the two cartilages. The result is a drooping of the nasal tip and the prelongation of the nose. The position of the lobular cartilage becomes more perpendicular (Figs. 2, 3)

In histological specimens this fragmentation can be easily seen. The perichondrium and the connective tissue enters into the cartilage splitting it little by little in two pieces which are connected only by connective tissue The cartilage and the connective tissue show in advanced age the same changes as in other parts of the body (Figs. 3 4 5)



Fig. 3 Lobular cartilage of 45-year-old man The perichondrium and the connective tissue invade the cartilage and cause its fragmentation. He + E, 30.



Fig 4 The lobular cartilage of a 60-year-old man. The fragments of the cartilage are connected only by connective tissue. He + E, 15

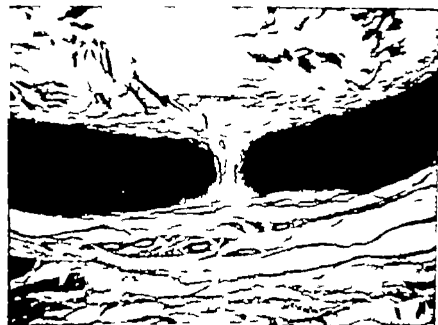


Fig 5 The lobular cartilage of a 65-year-old man with complete fragmentation: between the fragments there is connective tissue. PAS, 15

RESUMÉ

L'abaissement de la point du nez est la conséquence des changements de la forme et de la structure des cartilages de la point du nez. Chez des gens jeunes ce cartilage est convexe et résistant. Chez les per sonnes agées il devient plat et segmenté ce qui ésulte en un déplacement du cartilage vers le bas et une augmentation de sa hauteur

ZUSAMMENFASSUNG

Die Verlängerung der Nasenspitze im Alter ist die Folge der Veränderungen der Form und der Struktur der Spitzenknorpeln. Diese Knorpeln sind bei jungen Individuen konvex und widerstandsfähig. Im Alter wird der Knorpel gestreckt und segmentiert was eine Verlängerung des Knorpels in cranio-caudaler Richtung und eine Verlagerung derselben nach unten zu Folge hat.

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DISCUSSION

J Willemot When you showed pictures of human profiles you spoke about "nasolabial" angle and not about "septolabial" angle Might there not be a big difference between these two angles?

J Krmpotić-Nemanić (Reply) to Mr *Willemot*. By saying "nasolabial" angle, which expression is more common, I meant the "septolabial" angle.

Acta Otolaryng 71 282–287 1971

PHYSIOLOGY OF NYSTAGMUS

T Fukuda and T Tokita

From the E.N.T Department of Gifu Medical School, Gifu Japan

Abstract. We felt it necessary to record not only eye movements but also the movements of the head in order to study the physiology of nystagmus, therefore we constructed a telemeter to carry out these tasks simultaneously By the use of this telemeter we established that nystagmus is a very important motor reflex which can be observed in human actions and behaviour.

We would like to demonstrate some recent interesting findings on nystagmus during voluntary bodily movement as recorded by means of a radio-telemeter The present telemetry system was designed to record not only eye movements but also movements of the head in order to investigate the relationship between the movements of these two parts of the body Now we shall demonstrate our findings as revealed by the simultaneous graphic recording of eye and head movements.

As is shown in Fig. 1 electrodes are applied to the canthus regions on both sides of the head in order to record eye movements. Three accelerometers are attached to a helmet, one on the top, one on the right side and remaining one at the rear Three accelerometers are able to record lateral, anterior posterior and vertical movements respectively Channel 1 records horizontal eye movements. Channels 2, 3 4 record the degree and direction of accelerations of the head in three axes, i.e. lateral, anterior posterior and vertical axes as shown in Fig. 2.

When a subject began to walk, spikes appeared in Channel 4 indicating vertical head movements and, on turning around or changing the direction of walking, brisk eye nystagmus occurred as is seen in Channel 1 of Fig. 3. However when a subject undergoing drill changes direction on command, such multiple nystagmic movements do not occur one change in direction being accompanied by only one nystagmus is clearly shown in Fig. 4

Here you see a rotation which is called "pirouette" in ballet terminology One rotation was accompanied by one nystagmus, and the nystagmus is not only a movement of the eyes but also a movement of the head. Two rotations of the same turn evoked two nystagmic movements as shown in Fig. 5 The eye movements are indicated in Channel 1 and the horizontal head movements in Channels 2, 3 It is important to note that two nystagmic movements of the eyes are accompanied by two nystagmic movements of the head, and the head movements precede eye movements.

Now a ballet turn which is called "tour de chaines" is shown. This turn is performed by both a skilful ballet dancer and a novice. The former showed synchronous nystagmic movements of the eyes and head as is shown in Fig. 6, whereas the latter exhibited very erratic and irregular head and eye movements. It is important to notice the irregular vertical movements of the head which is shown in Channel 4

Now you still observe the eye movements of various subjects during a skating spin. The graphic record (Fig. 7) of a well trained skater showed many small but regular nystagmus during a spin. The graphic record of a moderately trained subject showed larger amplitude but


Fig 1 Radiotelemeter to record both eye and head movements simultaneously

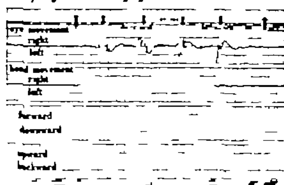
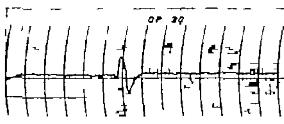


Fig 3 Brisk nystagmic movements of the eye on turning the direction of walking in channel 1


Fig 4 One change in direction undergoing drill was accompanied by only one nystagmus

less frequent nystagmus, whereas the record of a novice showed very irregular and erratic nystagmus. It is very interesting to note that marked eye nystagmus was clearly evidenced during rapid turnings of the spin.

Next, we would like to show the nystagmus of a skilful ballet dancer during and after turns in which he was instructed to keep his eyes open and then closed. As you see in Fig. 8, when his eyes remained open, no eye movement could be observed after he finished the turn, in other words, no postrotatory nystag-

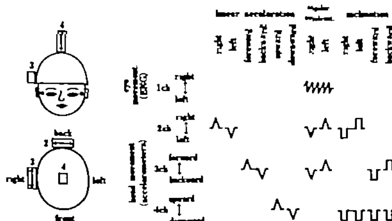


Fig. 2 Records of 4 channels.

Acta Otolaryng 71 282-287 1971

PHYSIOLOGY OF NYSTAGMUS

T Fukuda and T Tokita

From the E.N.T Department of Gifu Medical School, Gifu, Japan

Abstract. We felt it necessary to record not only eye movements but also the movements of the head in order to study the physiology of nystagmus therefore we constructed a telemeter to carry out these tasks simultaneously By the use of this telemeter we established that nystagmus is a very important motor reflex which can be observed in human actions and behaviour

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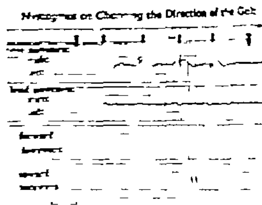


Fig. 3. Beat nystagmic movements of the eye ... turning the direction of walking in dance.

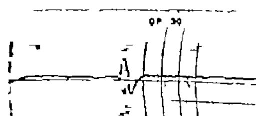


Fig. 4 One change in direction undergone, ... accompanied by only one nystagmus.

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Next, we would like to show the nystagmus of a skilled ballet dancer during and after turns in which he was instructed to kee... open and then closed. As you see ... when his eyes remained open, no eye ... ment could be observed after he ... turn. In other words, no postrot...

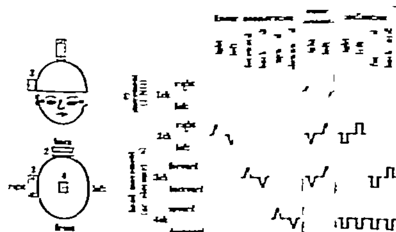


Fig. 2. ...

Ten ... during ... t, nys- ... rred ... char... one ... a ballet ... dancer de... 10 In ... eye moveme... the eyes ... whereas ... were neither

mus occurred. Then he was asked to close his eyes, after finishing the turn. Many beats of nystagmus were recorded for a long time after closing his eyes. This record is in clear contrast to the one with his eyes open and this fact was reported by Dr Collins and others (Collins, 1966)

Fig. 9 shows some interesting findings on nystagmus of the same dancer after he had been rotated passively 10 times in 20 sec in a rotating chair instead of turning actively through his own efforts. His eyes were open. After the rotation, his eyes showed no nystagmic movements. Here you can see brisk eye nystagmus occurred during the rotation, but once the rotation ceased, eye movement was scarcely detectable as you see in the recording. When a physically untrained person (a normal adult) was rotated in an identical manner a marked postrotatory nystagmus could be seen. Another characteristic phenomenon observed in the skilful ballet dancer is single large nys-

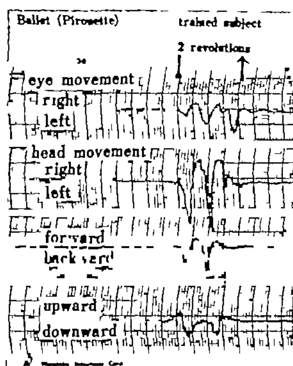


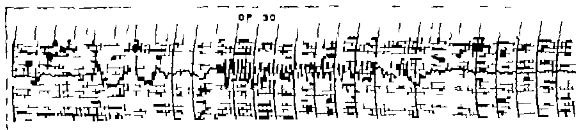
Fig 5 Two rotations in a "pirouette" were accompanied by two nystagmic movements of the eye as well as of the head.

Ballet (Tours chaînés)

Trained subject

Beginner

Fig 6 Nystagmic movements of the eye and of the head caused by rotations of "tour de chaînes" of a trained subject and of a beginner



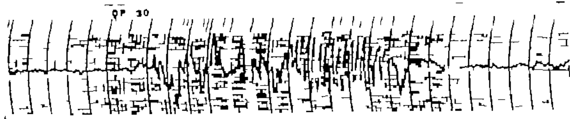




Fig. 7. Graphic records of eye movements during a skating spin.

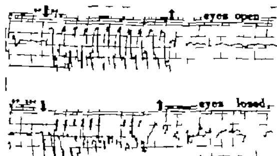


Fig. 8. Records of nystagmus during and after active rotations of skilful ballet dancer with eyes closed and then open.

tagmus accompanied by each rotation. Ten nystagmus movements were observed during the ten rotations. With a control subject, nystagmus of inconsistent amplitude occurred irregularly during rotation in a rotating chair.

Finally we would like to mention some differences between a skating spin and a ballet turn. Here you can compare a "tour de chaînes" with a skating spin in Fig. 10. In the former, the eyes and head show unison coincident movements. In the latter, the eyes showed brisk nystagmic movements whereas the insignificant head movements were neither nystagmic nor rhythmic.

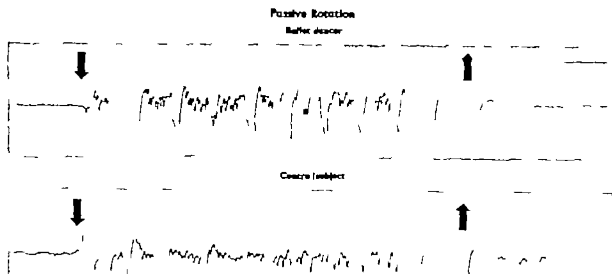


Fig 9 Records of nystagmus during and after passive rotations on a rotating chair of the same skilful dancer and of control subject.

Ballet
Tours chaînés

Skating
Spin

Fig 10 Records of eye and head movements of a "tour de chaines" in comparison with records of a skating spin.

Thus it is concluded that the nystagmus is an essential physiological factor of our daily locomotion. Human beings have learned to control this reflex phenomenon nystagmus" ingeniously and to utilize it for the smooth per formance of our bodily movement: therefore, nystagmus is one of the most important and essential motor reflexes which can be observed in human daily locomotion and behaviour

ACKNOWLEDGMENT

This paper is a synthesis of large number of ex perimental studies. The investigations were carried out by Dr T Tokita M.D with the collaboration of Dr T Watanabe M.D Dr M. Ogushi, Dr H. Miyata M.D Dr M. Fujigaki M.D., Dr T Kobayashi, Dr T Nagata, Dr K. Kat M.D., Dr Y Kato, Dr T Hibi, Dr R. Shimada, Dr T Suzuki, Dr T Taguchi and Dr Y Hayano, all of them are members of the E.N T Department of the Gifu Medical School.

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DISCUSSION

R. Hinchcliffe: Has Mr Fukuda employed this method in the investigation of patients complaining of vertigo? The potentialities are obviously great.

J D Hood. Mr Fukuda is to be congratulated upon his excellent film which is both stimulating and instructive. Although it raises many issues there is one to which I should like to draw particular attention namely the demonstration that following upon the cessation of a pirouette ballet dancer develops brisk nystagmus with closed eyes. Similar results to these have also been reported by Collins in the case of ice skaters. There is however an essential difference between the two because whereas an ice skater invariably keeps his head in rigid conformity with his body during a spin, a ballet dancer in the course of a pirouette fixes his gaze upon some stationary object and each complete rotation of the body is followed by very rapid 360° turn of the head when the object is refixated. It is commonly held that this manoeuvre eliminates the post-rotatory stimulus that would otherwise result and consequently accounts for the bsence f vertigo. I myself have always questioned this argument because if it were true then it is difficult to account for the fact that ballet dancers habituate since they would never be subject to habituating stimulus.

Mr Fukuda's excellent film now shows quite clearly that post-rotatory stimulus does exist despite the rapid rotation of the head and it now seems clear that this is the source of the habituating stimulus.

T Fukuda (Reply) to Mr *Hinchcliffe:* Cordial thanks for your suggestion. The eye and head movements of patients suffering from vertigo are not yet investigated. I hope to have a chance to report the results obtained in the near future.

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THE INFLUENCE OF UNILATERAL LABYRINTHECTOMY ON ORIENTATION IN SPACE

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(Received August 25 1970)

Abstract. A simple technique is described for the determination of the visual vertical and horizontal. In order to determine the influence of exclusively vestibular factors on spatial orientation, patients were tested prior and in the days following unilateral therapeutic labyrinthectomy Unilateral labyrinthectomy in patients operated upon for Menière's disease or cochleo-saccular degeneration introduces a marked deviation of the visual vertical and horizontal towards the operated side, which varies with the individual but is less pronounced in the group of patients operated upon for cochleosaccular degeneration. The origin, significance, and clinical applications of the phenomenon are discussed.

The influence of labyrinthine stimulation on space perception has been recognised for some considerable time since Purkyne in 1820 first described the apparent tilting of the environment, during the course of circular motion of a merry-go-round. Mach (1875) by his experimental studies distinguished between an objective vertical in the direction of gravity and a subjective vertical experienced by a subject undergoing circular rotation which is dependent upon the resultant centrifugal force and the force of gravity Kreidl (1892) noticed that deaf-mutes who did not show any eye movements on rotation, also experienced no apparent displacement of the vertical during circular motion and explained both deficiencies by the absence of labyrinthine function. The difficulty is, however that the undergoing of a movement like circular rotation stimulates not only labyrinthine receptors exclusively but also the proprioceptive system. Graybiel et al. (1968) separated the different factors by comparing the behaviour of normals and subjects with defective labyrinths in the normal situation and when immersed in water Their equipment, however is not available for clinical use and the method would be unsuitable for studying the influence of unilateral labyrinthine stimulation alone (Graybiel & Niven, 1953) A possible alternative in the study of the influence of exclusive labyrinthine factors upon spatial orientation is afforded by testing patients who had to undergo labyrinthectomy for therapeutical purpose pre and postoperatively The following system was used as an indication of the influence of labyrinthine loss on the perception of visual space

METHOD

The experimental technique is shown in Fig. 1

The patient was seated comfortably in a chair fitted with a head rest and arms, the head remaining unrestrained. From a distance of 180 cm (6 ft.) he viewed a blackboard which carried at its centre an illuminated rod 50 cm (1 ft. 8 ins.) long which could be rotated in the frontal plane. With the right hand, the patient was able to rotate the rod in a ise anti-clockwise direction by a

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Fig 1 By turning a circular knob an illuminated line in the dark is adjusted to the subjective visual horizontal and vertical. The line in vertical position at the beginning is turned clockwise (1), anti-clockwise (2) anti-clockwise (3), clockwise (4). This series repeated five times gives 20 values.

and-pulley drive The controlling knob was circular to exclude any spatial clues. Initially the patient was asked to rotate the rod from the vertical position to the horizontal position, then back to the vertical starting position and vice versa. After each positioning, the observer read the deviation in degrees (+ clockwise deviation, − anti-clockwise deviation) The extent of any error was concealed from the patient and the position of the rod was not corrected before the following move. Each se uence consisted of two clockwise and two anti ckwise rotations, providing two determinations of the horizontal and two of the vertical. This sequence was repeated five times, giving a total of 20 readings.

To familiarise the subject with the test procedure, the first five sequences were carried out in a lighted room but in the second part, the same five sequences were repeated in a completely darkened room (a small light being switched on and off after each determination to read and note the degree of deviation) Every patient thus made 20 determinations in a lighted room, and 20 determinations in the dark.

All experiments were carried out under the same conditions and by the same observer The time involved was approximately 30 min for all 40 determinations.

RESULTS

No significant difference was found between deviation of the horizontal and the vertical. If a deviation of the horizontal was present, then a deviation of the vertical in the same direction and of more or less the same magnitude also occurred. For simplicity therefore, the average value of all 20 determinations carried out in the light and the dark was separately calculated and shall henceforth be referred to as the mean deviation.

The mean deviation for the observations carried out in the light was at no time more than ±1 degree for all the subjects, and therefore the values for this part of the study are not recorded. They served to ensure, however that the subject had understood the experiment and was mentally and physically capable of carrying it out.

In the dark, normal individuals scored a mean deviation of about ±1 degree, and single values did not exceed ±2 degrees. Neal (1926) has shown, that this small size of error for the visual localisation of the vertical in the dark does not increase, when repeating the task over a prolonged period. For this reason a mean deviation in the dark of more then ±2 degrees was considered to be pathological. This is in agreement with similar figures arrived at by

Table I. *Patients operated for Menière's disease*

Symbol	Age	Sex	History	Hearing	Previous operations	Side affected
●	61	♂	Attacks for 9 years helped for 3 months by saccus decompression	R. hearing loss of 70 dB L. hearing loss of 20 dB	1965 R saccus decompression	R
△	43	♀	Very bad attacks for 3 years	R. normal L. hearing loss of 60 dB	—	L
○	42	♀	Attacks for 12 years improved for 3 years after ultrasonic destruction	R. hearing loss of 60 dB L. normal	1964 R ultrasonic destruction	R
■	46	♀	Attacks for 8 years worse recently	R. normal L. hearing loss of 60 dB	—	L
□	47	♀	Attacks for 14 years worse recently	R. normal L. hearing loss of 60 dB	—	L
	54	♀	Attacks for 9 years worse over last year	R. hearing loss of 60 dB L. normal	—	R

Bender & Jung (1948) using a different experimental system.

SUBJECTS

Six patients who had to undergo labyrinthectomy for advanced Menière's disease and 5 patients who underwent labyrinthectomy because of cochleo-saccular degeneration were tested on the evening before operation and during their post-operative period. The clinical details of our subjects are listed in Tables I and II.

In the patients suffering from Menière's disease, the operative indication was based on the severity of the attacks, the prolonged fail-

Table II

Symbol	Age	Sex	History	Hearing	Caloric test	Side affected
□	40	♂	L deafness since childhood probably mumps. Since 2 years attacks of vertigo	R. normal L. complete deafness	30° L NIL 30° R 2' 44° L NIL 44° R 1 50 20° L 1'50	L
●	31	♂	R deafness for 11 years of unknown origin. Since 14 months attacks of vertigo	R. complete deafness L. normal	30° L 2'10 30° R 1 50 44° L 2 10 44° R 1'50	R
△	17	♀	R deafness since mumps infection at the age of 6 years. Since 18 months tinnitus in R ear giddy spells and sickness	R. complete deafness L. normal	— — —	R
○	30	♀	R deafness since childhood. Attacks of vertigo for 4 years	R complete deafness L normal	30° L 1 15 30° R minimal 44 L 1 45 44 R minimal	R
■	19	♀	L deafness since childhood. Attacks of vertigo for 2 years	R normal L complete deafness	30 L 2'05 30 R 1 10 44 L 2'30 44 R 1'25	L

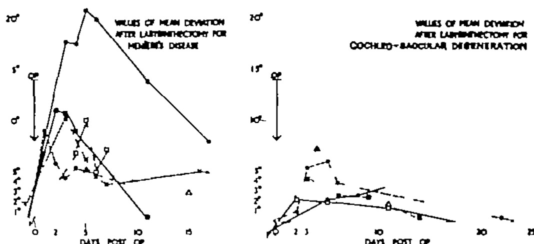


Fig. 2 For convenience all patients are considered to have undergone a labyrinthectomy on the right side. The direction of the deviation is therefore represented as + when directed towards the lesion and as − when directed towards the opposite side.

ure of medical treatment and the already severe hearing loss. In all cases, the disease was unilateral The term cochleo-saccular degeneration (Schuknecht et al., 1965) was used to describe a condition occurring in patients, who had a complete unilateral hearing loss, often in early childhood due to a virus infection. It is thought that in certain cases, although the cochlear function has been completely destroyed the labyrinth as the phylogenetically organ, is more resistant and although aged has not lost its function completely patients with complete unilateral deafness suffer from attacks of vertigo, it could be said to be due to this impaired but not inactive labyrinth even *if it is difficult to explain* why giddiness occurs sometimes so much later than the original affection. Proof that there is still some labyrinthine function is obtained by the presence of a response to caloric stimulation by the Fitzgerald-Hallpike technique (1942) *In the absence of a response, the affected ear* was further irrigated with water at 20 C for 1 min. If some labyrinthine activity could be demonstrated, labyrinthectomy was advised.

There is practically no contra-indication to a labyrinthectomy in these patients and the benefit they had seems to justify the operation, in retrospect. In all patients a transtympanic labyrinthectomy (Schuknecht, 1957 Ariagno 1964) was performed and special care was taken to remove not only the membranous canal but also the utricle.

RESULTS

The results of our patients are recorded in Table III and IV and illustrated in Fig. 2. All patients were tested for the first time on the evening before operation. None of the patients at this time had a complaint of vertigo and none showed spontaneous nystagmus on examination. Despite the fact that most subjects were apprehensive in view of the forthcoming surgical procedure, the mean deviation in all was well within normal limits.

After labyrinthectomy the patients showed the usual well known symptoms and disabilities, including spontaneous nystagmus with the rapid phase towards the normal side, unsteadiness, tendency to fall towards the operated side, vertigo and sickness aggravated by head movements. These disturbances were more violent in patients operated upon for Ménière's disease than for cochleo-saccular degeneration On the 2nd or 3rd postoperative day most of the patients were able to perform the test. Although they were not able to walk or stand on their own, they could sit and so carry out the test without outside help They found the task more difficult than normal sub-

Table III *Patients operated for Menière s disease*

Symbol	Pre-op.	Post-op. Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Week 2	Week 3	Week 4	Week 5	Week 6
● M.D	−1.9	+9 1	+5.8	+4.3	+5 4				+3	+0.4			
H.	−2.1	+9 4	+6.1	−4.3	+5				+3.1	+0.2			
V	−1.6	+8.8	+5.4	+4.3	+5 6				−2 8	−0.5			
S.N	—	+ + +	+ + +	+ +	+ +				+				
△ M.D	+0.4	Very giddy				−5 1			−2.9				−1.2
H.	+0.4					−5 7			−3.2				−1.5
V	+0.5					−4.5			−2.5				−0.9
S.N	—					+ + +			—				—
○ M.D	−1 4			+17.9	+17 7	+21.5	+20.3		+13.9	+7.9			
H.	−1 7			−17.8	+17.2	−21 4	+20.3		+13.9	−7.9			
V	−1.1			+18	+18.2	−21 5	−20.4		+13.8	+8			
S N	—			+ + +	+ + +	+ + +	+ + +		+ +	+ +			
■ M.D	+0.6		−11	−10.7	− 8.9				−0.3				
H.	0.5		−11.9	−11 7	−10.4				−0.3				
V	+0.7		−10 1	− 9 7	− 7 4				0 0				
S N	—		+ + +	+ + +	+ +				—				
□ M.D	+0.8				−6.9	−10.1	−4 7	−7.3					
H.	+0.7				−7.5	−10 7	−5 7	7 7					
V	+1				−6.3	− 9 6	−3.6	−6.9					
S.N.	—				+ −	+ + +	+ +	+ +					
M D	+1.3			+10.6		−6.2	+4.3	+3 7		−5	−3.9		
H.	+1.2			+10.4		6 1	+3.5	+3.3		−4 6	−3.8		
V	+1.3			10.7		−6.3	−5 2	4		+5.3	−3.9		
S N	—			+		+	−			+	−		

Abbreviations. M.D = Mean deviation, H. mean value of 10 determinations of the horizontal, V = mean value of 10 determinations of the vertical S.N = spontaneous nystagmus (+ = first, + + = second, + − third degree).

Table IV *Patients operated for cochleo-saccular degeneration*

Symbol	Pre op	Post-op. Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Week 2	Week 3	Week 4	Week 5	Week 6
□ M.D	+1.6		2.2			−2			1 4	0 0			−0.2
H.	+1		−3			−2.3			−1.8	0.1			−0.4
V	+2.1		−1 4			−1 7			−1.0	−0.1			0.0
S.N	—		+			+			−	−			−
● M.D	1.2	Very giddy				+2..			2.6	−3 6	3.2	3	
H.	−1 7					+1.6			2 3	3.1	+3 1	2.2	
V	−0.7					+2.7			+3	+4	+3.3	−3.7	
S.N.	—					+			−	+	−		
△ M.D	0.1	Very giddy			7.2				−1 7			0.8	
H.	−0.6				−7..				1.3			0 7	
V	0.4				7 1				2.1			1.0	
S.N.	—												
○ M.D	+0.7		+0.9	5		6	3.8				0 0		
H.	1		+1.6	5 7		7	5				−0 8		
V	0.4		+0.2	4 4		5	2.6				0.7		
S N.	—												
■ M.D	0.3			4 1			5		2.5	−0.4			
H	−0 6			3 6			2 6		−3	0.3			
V	0 0			4 6			2 4		−2	−0.5			
S N.	—									−			

Abbreviations, see T ble III.

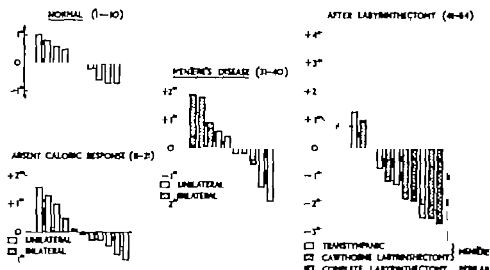


Fig 3 Each column represents the value of the mean deviation for one patient.

jects, but their mean deviation remained within normal limits as long as the test was performed in the illuminated room, so that visual cues were available.

In darkness most patients complained that the line appeared to move or was unsharp or bending in different directions, but after over coming these initial difficulties they performed well and were constant in their settings. All were on a variable dose of Prochlor ("Stemetil") and Dimenhydrinate amamine") as part of their post-operative treatment. They stayed normally in hospital for a week and were tested during this time as often as their condition and facilities allowed. Regular follow-up in short intervals was difficult after discharge because of the travelling involved.

It is evident that unilateral labyrinthectomy introduces a very marked deviation of the visual vertical and horizontal in the dark, which is always directed towards the operated side (Fig. 2). The deviation is most marked in the first week following operation and tends to regress rapidly during the 2nd and 3rd postoperative week. No significant difference could be found between the deviation of the horizontal and the deviation of the vertical.

The deviation is much more pronounced in patients operated upon for Menière s disease than in those operated upon for cochleo-saccular degeneration. As already mentioned a similar difference between the two groups was also noticed for other postoperative disturbances. Although the majority of patients follow a similar pattern, there are large individual dif ferences concerning the extent of the pathological deviation and this is more evident in the group of patients operated upon for Menière s disease.

In summary unilateral labyrinthectomy in patients operated upon for Menière s disease or cochleo-saccular degeneration introduces a marked deviation of the visual vertical and horizontal towards the operated side which varies with the individual but is less pronounced in the group of patients operated upon for neurolabyrinthitis.

DISCUSSION

Earlier studies (Friedmann, 1970) have shown that patients suffering from peripheral vestibular disease when tested in the non acute stage, score a normal mean deviation (Fig. 3).

In addition the vast majority of patients who have undergone labyrinthectomy for Menière's disease compensate fully and their subsequent mean deviation remains within normal limits. In a few patients, however especially when operated upon for perilabyrinthitis (Cawthorne, 1957) the mean deviation was found to be pathologically raised between 3–4 degrees and directed towards the operated side, even when tested at least one year after operation. The results of animal experiments (Gernandt & Thulin, 1952 Precht et al., 1966) have shown that after unilateral labyrinthectomy or vestibular nerve section, the spontaneous neural activity of the ipsilateral vestibular nucleus will be severely reduced, whereas the activity of the contralateral nucleus will remain unaffected, therefore a state of central imbalance is created. Reactivation of the de afferented vestibular nucleus probably through the reticular system takes place in the weeks following operation, and so leads to a compensation of the peripheral defect at a central level. In some patients however this central compensation remains incomplete (Spiegel & Demetriades, 1925 Fluur 1960 McCabe & Ryu, 1969) The production of an abnormal deviation by labyrinthectomy seems to be therefore another manifestation of tonus imbalance at the level of the vestibular nuclei. It is a temporary phenomenon which regresses post operatively and disappears completely in most cases.

It is tempting to explain the occurrence of a deviation in terms of a simple rotation of the eye ball and the retina provoked by a tonus difference of the eye muscle. However Fischer (1927 1930) examining the Aubert phenomenon—the fact, that a vertical illuminated line, when observed with the head tilted to one side appears to be tilted to the opposite side (Aubert, 1861)—could find no simple relationship between the degree of counter-rolling of the eye balls and the angle of the apparent tilt of the vertical. In the same way Vogelsang (1961) has shown that the opto-gyral illusion does not depend on the presence of nystagmus. Dishoeck et al. (1954) were unable to provoke any horizontal nystagmus in a patient suffering from bilateral abducens paralysis, whereas the opto-gyral illusion was of normal duration and direction. Similarly a case of brain-stem tumour although suffering from a vertical gaze palsy had a highly pathological mean deviation (Friedmann, 1970)

Obviously space perception is not only dependent on the localisation of an image on the retina, but it is also influenced by extra-retinal factors and based on an interplay of visual, proprioceptive and vestibular influences, which takes place in the brain-stem (Bender & Feldman, 1967) probably in certain neurons of the reticular formation (Duensing & Schaefer 1960) Whereas visual stimuli are normally dominant as is evident from the finding of a consistently normal mean deviation in the lighted room, vestibular influences will become important after the exclusion of visual cues.

The multitude of factors involved in space perception may explain the large differences in our patients. It seems likely that people with a long standing and probably constant loss of labyrinthine function resulting from such conditions as cochleosaccular degeneration will already have developed compensatory mechanisms before operation, and so be less affected by labyrinthectomy Patients with Menière's disease—a fluctuating condition which appears later in life—may have developed less compensation and therefore react more violently to vestibular imbalance Another possibility is that patients suffering from Menière's disease show an exaggerated reaction to any vestibular stimulus. In their study of 77 patients who underwent labyrinthectomy for Menière's disease, Simonton & Sciarra (1958) could find no preoperative criterium to which the resultant postoperative disability could be related. It seems likely that even with improved methods of measuring labyrinthine function, we will not be able to forecast the disturbance resulting from the loss of vestibular function, because of the multitude of factors involved and the large individual differences, when reacting

I

II

Fig 4 ENG· DC recording with eyes open in darkness looking straight ahead. (I) Recording from 19 Nov 1968 marked spontaneous nystagmus is present, note the tendency of the eyes to deviate in direction of the slow component. (II) Recording from 17 Dec. 1968, absence of spontaneous nystagmus confirmed by ENG

them. If we are therefore interested in knowing how vestibular pathology alters the behaviour of patient, it will not be enough to measure the sensitivity of the peripheral vestibular organ alone—which will remain at zero after labyrinthectomy—but we will also have to measure functions which necessitate the interaction of the vestibular system with other systems, in order to be able to assess the development of compensatory mechanisms. In this respect the measurement of spatial orientation in the k could well prove of importance in provid -mplementary information.

CLINICAL APPLICATION

As has been indicated earlier the test is not revealing in the diagnosis of established peripheral vestibular lesions, because full compensation will have taken place. Unfortunately we were unable to test patients suffering from acute labyrinthine lesions. One patient however suffered an acute attack of Menière's disease when attending the Outpatient Department. His history and examinations are described as follows:

E. C. 45 years old, had been suffering from attacks of vertigo during the past 4 months between which he was perfectly well. The attacks occurred about twice a week and since the onset he had noticed left-sided tinnitus and deafness. The onset of the attacks coincided with his mother's fatal illness during which time he had a good deal of extra worry

On examination he appeared a fit and healthy man. Nothing abnormal was to be seen in the ears, nose and throat.

Cochlear function: Left—some deafness was present for all frequencies within the range 200–8 000 c/s, severe for the high tones. Loudness recruitment was complete Right—normal. Caloric test. Absence of the left responses, slight directional preponderance to the right of the right caloric responses. No other vestibular abnormalities.

During the course of the morning the patient told us that he felt as though an attack was coming on and a persistent nystagmus to the right with a rotatory element developed during the examination. Electronystagmographic studies were arranged in the afternoon. By this time the nystagmus to the right had disappeared being replaced by a third degree vestibular nystagmus to the left, which was enhanced with both eye closure and darkness (Fig. 4 I) indicating a peripheral vestibular lesion (Hood, 1967) Immediately after the electronystagmographic recording the patient carried out the described test of spatial orientation.

A markedly pathological mean deviation was found of *+18 degrees* The single values were fairly constant and no difference was found between the horizontal and the vertical.

A diagnosis of *left Menière's disease* was made and medical treatment was prescribed.

When the patient was seen again 1 month later he stated that he had suffered three further attacks but that he had been improving recently When examined, there was no spontaneous nystagmus (Fig. 4 II) and his mean deviation was with +0.5 degree well within normal limits.

It is interesting that this patient when tested during an acute attack of Menière's disease showed a highly abnormal mean deviation, but

directed to the side opposite the affected labyrinth. This is in contrast to the findings following labyrinthectomy and would indicate that the symptoms in acute Menière's disease are due to vestibular imbalance created by hyperactivity of the diseased labyrinth and that the loss of function is only secondary. Although this was a single observation it does however serve to demonstrate the usefulness of testing spatial orientation in acute vestibular lesions.

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Grateful acknowledgment is made to the late Sir Terence Cawthorne, Dr M. R. Dix and Mr Spencer Harrison who made available the patients upon whom this study is based. The operations were performed by the late Sir Terence Cawthorne and Mr Spencer Harrison and we wish to express our gratitude to them. Special thanks are due to Dr J. D. Hood under whose direction this work was carried out and to the various members of his staff for their technical assistance.

We would also like to thank the editor of Brain for the permission to reproduce Figs. 1 and 3.

ZUSAMMENFASSUNG

Eine einfache Technik zur Bestimmung der visuellen Vertikalen und Horizontalen ist beschrieben. Patienten, in denen das Labyrinth aus therapeutischen Gründen zerstört wurde, wurden unmittelbar vor und in den Tagen nach der Operation getestet, um den Einfluss von ausschliesslich vestibulären Faktoren auf die Orientierung im Raume zu messen. Die Patienten wurden wegen Menière'scher Krankheit oder cochleo-sacculärer Degeneration operiert. Die unilaterale Labyrinthzerstörung bewirkte eine markante Abweichung der visuellen Horizontalen und Vertikalen zur operierten Seite. Das Ausmass der Abweichung unterliegt starken individuellen Schwankungen und die Abweichung ist bedeutend schwächer bei den Patienten, die für cochleo-sacculäre Degeneration operiert wurden. Entstehung und Bedeutung dieses Phänomens werden diskutiert und die klinische Anwendung an einem Beispiel erläutert.

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VESTIBULAR NYSTAGMUS—A DIFFERENTIAL REACTION

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Abstract Galvanic polarization was applied to 20 adult spinalized cats in order to study the interplay between the two labyrinths during different stimulation conditions. The results have shown that it is the difference in activity between the two reflex arcs which determines the direction and intensity of the reaction i.e., the vestibular reflex arcs function in the same way as a differential amplifier

In 1892 Ewald advanced a theory that the semicircular canals react on both ampullopetal and ampullofugal endolymph movements. This theory has later been verified in neurophysiological studies by Löwenstein & Sand (1940) who established that the horizontal semicircular canal at rest has a remarkably constant activity which can be caused to increase or decrease during ampullopetal and ampullofugal endolymph movements, respectively These results have been confirmed by recordings both from the vestibular nerve (Gernandt, 1949 Ledoux, 1956) and from the vestibular nuclei (Adrian, 1942 Gernandt & Thulin, 1952 Eckel, 1954 Duensing & Schaefer 1958) Thus peripheral sensitivity in the two directions of movement of the cupula has been called bidirectional sensitivity and only implies that the electric activity increases when the cupula moves in one direction and decreases when moved in another On the other hand, simultaneous recordings from the right and the left vestibular nuclei show that they have a constant electric activity at rest, equal on both sides (Eckel, 1954) During rotation of an experimental animal the activity increases in one nucleus and decreases in the contralateral. These results can be interpreted in two different ways and give rise to different questions with regard to nystagmus.

1 Are the responses in the nuclei only a propagation of the peripheral activity difference shown by Löwenstein and Sand, which should be capable of running the vestibulo-ocular reflex nystagmus in two opposite directions, i.e a monolabyrinthine bidirectional sensitivity?

2. Is nystagmus a result of a difference in the electric activity between the right and left reflex arc, i.e., the vestibular nystagmus is a differential reaction.

Fluur & Mellström (1970) have shown by monolabyrinthine stimulation in cats that the bidirectional sensitivity by no means signifies that it is possible to run the vestibulo-ocular reflex nystagmus in two opposite directions only by stimulation of one semicircular canal. That it is nevertheless possible after a certain time, to elicit nystagmus in both directions even in monolabyrinthine animals, is a finding described already by Bechterew (1883) and lately studied by McCabe & Ryu (1969) The phenomenon is dependent on centrally elicited compensatory mechanisms, implying the development of a new electrical activity in the vestibular nuclei on the labyrinthectomized side.

In order to study question 2, whether the vestibular nystagmus may be a differential re

very great difference in activity between the two sides and, consequently very intense nystagmus. Cold water irrigation, on the other hand, decreases the difference in activity down towards zero. When there is no difference in activity the patient has no nystagmus even for a short time after irrigation (Fluur 1960)

ZUSAMMENFASSUNG

Galvanische Stimulation ist an ?0 erwachsenen spinalizierten Katzen applitiert um das Zusammenspiel zwischen den zwei Labyrinten während verschiedener Stimulationsbedingungen zu studieren. Das Resultat hat gezeigt dass es der Unterschied in Aktivität zwischen den beiden Reflexbögen ist, der die Richtung und Intensität der Reaktion bestimmt, das heisst der vestibulare Reflexbogen funktioniert in derselben Weise wie ein Differentialverstärker

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ORGAN CULTURE OF THE MAMMALIAN INNER EAR

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(Received May 13 1970)

Abstract An organ culture technique that is 90% ffective has been established that will routinely allow the growth and development of the twelfth gestation day mouse inner ear The in vitro development lags behind the in vivo development but follows the pattern of in vivo development.

There are twenty publications documenting the in vitro development of the avian inner ear Fell (1938) reported the in vitro culture of isolated 4 day chicken embryo otocysts on plasma clots. Since Fell s report, investigators have communicated various techniques for the in vitro development of the fowl embryo otocyst.

Friedmann (1956, 1959 *a*, 1959 *b* 1961 1965 1968 1969) and Friedmann & Bird (1961 *a*, 1961 *b* 1967) have described the ultrastructure of the sensory and neural components of otocysts developed in vitro. McAlpine & Friedmann (1963) have reported a histochemical analysis of organ cultured otocysts.

Orr (1968) has investigated disassociation and reaggregation of chick embryo otocysts in vitro.

There is very little information defining the in vitro development of the isolated mammalian inner ear The three attempts to culture this inner ear are described in the following communications. Maximow (1925) was the first investigator to report the in vitro growth of the rudiments of mammalian embryos. He observed that otocysts contained in fragments of rabbit embryos did not undergo any changes. The otocysts gradually decreased in size and then degenerated. Lawrence & Merchant (1953) issued a report of the culture of the 9 day rat embryo otocyst on a plasma clot The cultures were maintained for 8 days by this technique. They report that there was no semicircular canal formation and that the cochlear duct started to form but did not coil. The cells that form the organ of Corti were observed to be present in early stages of development. Shambaugh (1956) investigated the effects in vitro of normal saline, Ringer solution and Gey balanced salt solution upon newborn-cat middle ear mucosa and the cells of Corti's organ. The tissues were placed in a Pomerant perfusion chamber and the fresh tissues were bathed in the respective solutions. Normal saline caused irreversible cellular damage After 1 hour Ringer solution caused patchy necrocytosis and Gey balanced salt solution had no detrimental effects.

The mouse embryo has been selected for the present investigation because many types of genetic, congenital deafness have been identified in the mouse (Deol, 1968). Abnormal ear development may be observed and possible causative mechanisms investigated in an in vitro environment after a routine organ culture method has been established.

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[1] This work is part f doctorate thesis that is to be submitted to The New York University Graduate School of Arts and Science.

[2] Health Research Council, City of New York, Career Scientist Award no. 1 589

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Orr (1968) has investigated dissassociation and reaggregation of chick embryo otocysts in vitro

There is very little information defining the in vitro development of the isolated mammalian inner ear The three attempts to culture this inner ear are described in the following communications. Maximow (1925) was the first investigator to report the in vitro growth of the rudiments of mammalian embryos. He observed that otocysts contained in fragments of rabbit embryos did not undergo any changes. The otocysts gradually decreased in size and then degenerated. Lawrence & Merchant (1953) issued a report of the culture of the 9 day rat embryo otocyst on a plasma clot. The cultures were maintained for 8 days by this technique. They report that there was no semicircular canal formation and that the cochlear duct started to form but did not coil. The cells that form the organ of Corti were observed to be present in early stages of development. Shambaugh (1956) investigated the effects in vitro of normal saline, Ringer solution and Gey balanced salt solution upon newborn-cat middle ear mucosa and the cells of Corti's organ. The tissues were placed in a Pomerant perfusion chamber and the fresh tissues were bathed in the respective solutions. Normal saline caused irreversible cellular damage After 1 hour Ringer solution caused patchy necrocytosis and Gey balanced salt solution had no detrimental effects.

The mouse embryo has been selected for the present investigation because many types of genetic, congenital deafness have been identified in the mouse (Deol, 1968). Abnormal ear development may be observed and possible causative mechanisms investigated in an in vitro environment after a routine organ culture method has been established.

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[2] Health Research Council, City of New York, Career Scientist Award no. 1 589

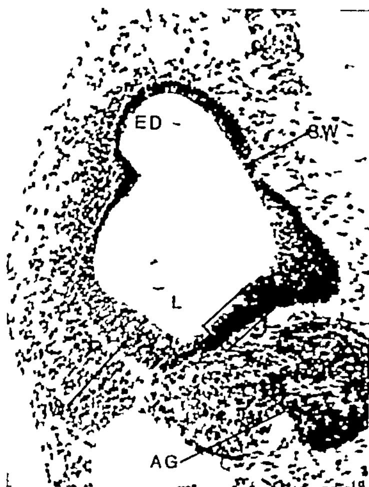




Fig 1 12th gestation day CBA J/CBA-J ...use embryo inner ear ( ) Otocyst ... 97 (*b*) Inferior wall of otocyst ... 410.

Abbreviations use

AG	Acoustl	L,	
BM	Basilar	MF	Figure
Cart	C	PL,	...tic
ED	Endol	SF	Epithel
ES,	E		...ar
IW			W



Fig. 2. 12th gestation day CBA J CBA J mouse embryo inner ear (*a*) Otocyst in vitro 0 hours, control. 92 (*b*) Inferior wall of otocyst. (*a*) 515

METHOD

CBA J/CBA J mice obtained from Jackson Memorial Laboratories are used. The onset of gestation is determined by the vaginal plug method. Male CBA J/CBA J mice are placed with female CBA J/CBA J mice in the evening. Females are inspected the next morning for a vaginal plug. The female mice are sacrificed on the twelfth day of gestation by cervical dislocation and the embryos removed but kept in the uterus which is then placed in a prewarmed Hanks balanced saline solution. The embryos are removed one at a time and both otocysts with associated mesenchymal and neural tissue are dissected from the embryo. The dissection



Fig 3 1_th gestation day CBA J/CBA J otocyst 4 days in vitro. (*a*) The developing semicircular canals and cochlear duct portion of the in vitro otocyst. 90. (*b*) The coiling cochlear duct portion of the in vitro developed otocyst. 97 ( ) Sensory epithe lium of the cochlear duct shown in (*b*). 500

is done in sterile Hanks balanced saline solution S) with the aid of a Leitz dissecting micro- , no 3 watchmaker's forceps and fine ungsten probes that are presharpened to a tip several microns in diameter

The excised otocysts are placed into a Petri dish containing prewarmed tissue culture medium This medium was a variable and the various types used are defined in the results. When all the otocysts are excised, several are randomly selected from the group and fixed as controls.

After the tissue is grown in organ culture, for varying amounts of time as specified in the results, they are processed for histology using the polyester wax technique of Ruben & Sid man (1967). Acrolein at a final concentration of 10% with phosphate buffer pH 7.2, is the fixative for 1 hour at room temperature The fixed specimens are washed in phosphate buffer pH 7.2. Dehydration with a 1 1 solution of methyl alcohol and methyl cellusolve is accomplished in 2 to 3 days. The dehydrated specimens are infiltrated for 3 hours in 90% polyester 400 distearate with 10% cetyl alcohol The organ cultures are imbedded and left at room temperature overnight, then stored at 4 C. Tissues are sectioned at 7 μm on a Leitz rotary microtome Sections are serially mounted on slides with 0 1% gelatin. Harris hematoxylin and eosin is the staining technique employed. The microscopic analysis rates the morphological changes and histological differentiation of the otocyst controls vs. specimens.

RESULTS

The first group of otocysts were cultured in Belco glass culture chambers. The chambers were incubated in a stationary position at 33 C. Either medium 199 with Hanks BSS or Eagle minimal essential medium (MEM) with Earle BSS was used as the liquid phase These media were supplemented with fetal calf serum as 30% of the total volume and lactalbumin hydrolysate as 0 25% by volume. The culture chambers were useful for microscopic examination of the cultures, but differentiation of the otocyst was not directly observed because the mesenchymal tissues proliferated rapidly and the specimen became too thick. The culture media were changed every 2 days. The specimens were fixed at 4 7 and 10 days in vitro. Fifty percent of 100 cultures maintained under the above conditions exhibited poor development with various degrees of central necrosis. Approximately 20% of the 50 cultures that were healthy displayed some differentiation beyond the development of the 0 hr controls. This was usually in the form of differentiation of the stratified cells of the ventral portion of the otocyst into early sensory epithelium.

The otocyst organ cultures in a second group were explanted into roller tubes with silicone stoppers and rotated in a roller drum at 1 rev min. The temperature of incubation was 33



Fig. 3 b–c. For text see Fig. 3 *a*.

or 37 C. The volume of medium in each tube was 1.5 ml with one otocyst per tube. The culture media were medium 199 with Hanks or Earle BSS, Eagle MEM with Hanks or Earle BSS and Waymouth medium MB752 1 Medium-199 with Hanks BSS was found to be the best medium when supplemented with lact albumin hydrolysate 0.25% of final volume and fetal calf serum, 30% of final volume An incubation temperature of 37 C proved to be best for development. Almost all specimens showed necrocytosis of different degrees. Approximately 50% of 180 organ cultures displayed marked necrocytosis and no mitotic figures. Some degree of advanced differentiation was observed in 30% of the 90 healthy cultures. The histological development observed was the formation of a presensory epithelium from the stratified ventral portion of the otocyst. In a few specimens there were major morphological changes the elongation of the endolymphatic duct, folding of the posterior wall to

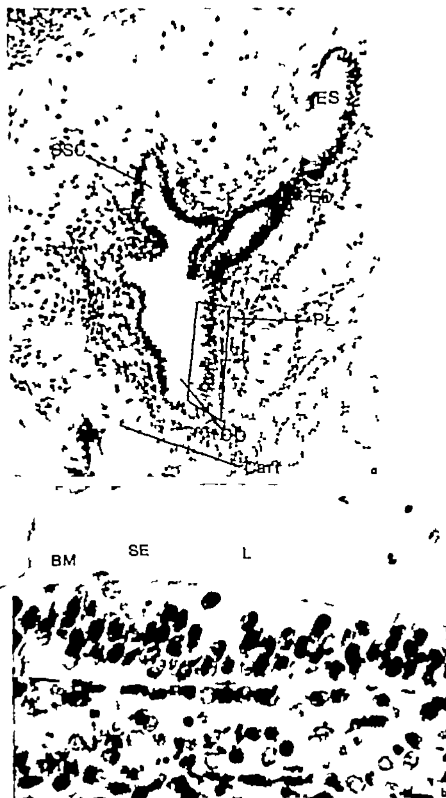


Fig. 4. 12th gestation day CBA J/CBA J otocyst 4 days in vitro. ( ) Organ culture specimen with developing semicircular canals and cochlear duct. 87. (*b*) Developing sensory epithelium of cochlear duct, (*a*) 495.

form the primordium of the semicircular canals and an elongation of the ventral wall to form a cochlear duct. This group of specimens with advanced differentiation was comprised of a total of 30 cultures the most advanced differentiation described above was seen in two specimens of the group

A third variation in technique was as follows. Forty otocysts were cultured in Leighton tubes with silicone stoppers at 37 C. Each tube contained 0.5 ml of nutrient media and four otocysts. The medium was changed every 4 days. We used medium 199 with Hanks BSS or Eagle MEM with Earle BSS. These media were supplemented with lactalbumin hydrolysate, 0.25% of final volume and fetal calf serum, 30% of final volume. This system was designed to study the effects of several otocysts in a small volume of nutrient medium, the theory being that if the otocysts were producing a substance that would diffuse into the medium this would act to produce a high concentration of these factors and perhaps yield more advanced differentiation in the developing otocysts. This did not occur the otocysts frequently fused with one another and most specimens exhibited extensive necrocytosis. These poor results were thought to have occurred because of any one, or a combination, of the following reasons: a depletion of the dissolved oxygen in the nutrient medium a depletion of essential nutrients of the culture medium, a concentration of toxic metabolites in the medium All of the above reasons can be ascribed to a single fault in the system, too small a volume of culture medium per otocysts.

The three basic techniques described, with approximately twenty other variations, led to a fourth generalized scheme for growing the mouse otocyst in vitro. The otocyst is explanted into Falcon plastic organ culture dishes. The temperature in the incubator is kept at 37.5 C and the cultures are stationary The environmental gas atmosphere of the incubator (Wedco) was varied Several gas compositions were tried: either 100% air free flow 100% air closed system or 5% carbon dioxide and 95 free flowing air The relative humidity was maintained at either 70% or 90% The optimal atmospheric condition was found to be 100% closed air system at 90% relative humidity The culture media were medium 199 with Hanks BSS or Eagle MEM with either Hanks or Earle BSS Fetal calf serum, 30% total volume, 0.25% lactalbumin hydrolysate and 0.01 mmole of sodium pyruvate per 1 ml of the culture medium were supplements. Extracts of chicken embryos and/or of mouse embryos were used to supplement the medium in a final concentration of 10% of the final volume The chick embryo extracts were prepared from embryos incubated either 8 to 10 days or 10 to 12 days. The mouse embryo extract was prepared using a combination of 12, 14 16 and 18 day gestation embryos. The explant was grown on either a stainless steel grid with lens paper coated with 1% Difco Ion Agar a stainless steel grid with a 45 μm APD Millipore filter or plain gelfoam sponge.

Medium 199 with Hanks BSS in an ambient air atmosphere of 90% relative humidity proved to be the best system. The medium is supplemented with 0.25% lactalbumin hydrolysate, fetal calf serum 30% by volume, 0.01 mmole of sodium pyruvate per 1 ml medium, and 10% chick embryo extract of 10 to 12 day chick embryos or 10% fetal mouse embryo extract. Gelfoam is a better growing surface than is Millipore filter The surface of the Millipore filter caused the death of cells in contact with it. It appears that the Millipore filter must be detoxified prior to utilization as a growing surface in vitro.

Approximately 90 cultures of a group of 100 were healthy with minute or no observable necrocytosis, using the system described above Almost all of the healthy cultures showed some degree of differentiation beyond the 0 hr controls. The most recent cultures have developed in 3 days in vitro to the equivalent of 1.5 to 2 days development in vivo. The endolymphatic duct elongated and formed an endolymphatic sac. The pars superior of the otocyst formed rudimentary semicircular canals. The pars in-

shown in the fourteenth gestation day specimen of Fig. 8

ZUSAMMENFASSUNG

Es wurde eine zu 90% effektvolle Methode der Organzucht entwickelt, die Wachstum und Entwicklung des Innerohres eines Maus am 12. Tag der Trächtigkeit routinemäßig ermöglicht. Die in vitro Entwicklung steht der in vivo Entwicklung nach, folgt jedoch dem Muster der in vivo Entwicklung.

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A STUDY OF THE CURRENT SPREAD ON ELECTRIC STIMULATION OF THE INDIVIDUAL UTRICULAR AND AMPULLARY NERVES

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Abstract In the cat, single vestibular nerve branches were electrically stimulated through implanted electrodes. Different combinations of extraocular muscles were activated by each of the single vestibular nerve branches, and there were three frequency-dependent contraction modes, i.e. the lateral-canal, the vertical-canal and the utricular type. It was shown that these could be utilized for detecting whether single or combined vestibular nerve branches were stimulated.

Our previous report (Suzuki et al., 1969 *a* Suzuki et al., 1969 *b*) demonstrated that single utricular nerve stimulation induced strong rotatory eye movements associated with slight vertical and horizontal shifts. It also showed that temporal summation of activities in the extraocular muscles during stimulation of the utricular nerve had a pattern different from that of other vestibular nerve branches.

For the studies in which single nerve stimulation is important, demonstration of selective single nerve stimulation is required. It is expected that stimulation of other nerve branches besides the intended branch will change the combination of activated eye muscles and also the mode of contraction of those muscles. The purpose of this paper is to demonstrate, from the contractions of the eye muscles, single or combined vestibular nerve branch stimulation.

This study was supported by Research Grant NB-06585 from the National Institute of Neurological Diseases and Blindness, US Public Health Service, USA and the Naito Science Foundation, Tokyo, Japan.

METHODS

Twenty healthy cats were used for this study Tracheotomy and high cervical transection were performed under general anesthesia which was then discontinued. The electrode implantation and experiments were carried out under local anesthesia. The cat was maintained quiet and warm under artificial respiration. This preparation was adequate for the study of eye movements and care was taken that the animals were alert or drowsy without any signs of pain or discomfort.

During electric stimulation of each vestibular nerve branch, changes in eye muscle tension were isometrically recorded with a transducer RCA-5734 Individual nerves were electrically stimulated with bipolar stainless steel electrodes of 50 μ diameter using square waves of 0 1–0.5 msec duration. Pulses were usually given in trains which consisted of 30 or 50 pulses separated by 0 6, 1 0 1 6, 2.5 4 0 6.4 10 0 msec. Each train was given at frequency of one per second. For studying the responses induced by different frequencies of pulses, the number of pulses in a train was held constant and pulse interval was changed. Accordingly pulse trains with short pulse separations were short, and those with long pulse separation were long. In order for convenient comparison of the differences in ocular muscle contractions, the curves of muscle tension increase in-



Fig 1 Serial horizontal sections of the left temporal bone in a cat. Abbreviations indicate each vestibular nerve root where the stimulating electrodes were implanted. *A* anterior semicircular canal nerve *L*, lateral semicircular canal nerve *U* utricular nerve *S* saccular nerve *P* posterior semicircular canal nerve. Approximate distances between pairs of sections of A and L, L and U U and S, and S and P are 400 μ, 400 μ 1 000 μ and 1 400 μ respectively

duced with different pulse trains were superposed.

oth liminal and supraliminal stimulations were used to observe differences in induced responses. Individual vestibular nerve branches run together in the nerve but are separated near the end organs of the canals or utriculus. Therefore, liminal stimulation is expected to excite a single nerve branch, while supraliminal stimulation might excite nearby nerves even with an adequately implanted electrode.

Serial sections of cats temporal bone were taken for histological studies from several successful experiments.

RESULTS

The vestibular nerve bifurcates peripherally in the internal acoustic meatus or in the bony capsule and afterward the branches diverge. The space between each of the vestibular nerve branches is largest where they end at their sensory organs, therefore the stimulating electrodes should be on those portions of the nerve. The distances between nerves at the end organ were measured in the reconstructed maps from serial sections of the temporal bone (see Fig. 1).

Excitation of a single vestibular nerve branch always induced the same reproducible mode of contraction in its primary and synergistic ocular muscles

As previously reported, liminal stimulation of the anterior posterior and lateral ampullary and utricular nerves produced their own characteristic patterns of contraction curves in each of the primary active ocular muscles (Suzuki et al., 1969 *b* Cohen et al., 1964) Thus when stimulating the utricular nerve, its primary muscles developed their maximum tension with high frequency stimulation, those of the lateral nerve developed maximum tension with low frequency stimulation and those of the vertical

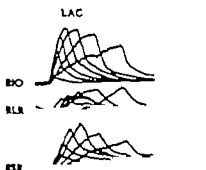


Fig 2 Eye muscle contraction during electric pulse stimulation of the anterior ampullary nerve. Increase in tension of the inferior oblique (*RIO*), lateral rectus (*RLR*) and superior rectus (*RSR*) of the right eye was induced by stimulating the left anterior semicircular canal nerve (*LAC*). Trains of 30 pulses with 0.3 msec width, 0.6, 1.0, 1.6 2.5 4.0, 6 0 and 10 0 msec interval and low voltage near threshold were used. Those tension curves were recorded isometrically and superimposed. The modes of tension increased in those three muscles which were similar to each other although tension curves of RLR, which is the tertiary muscle, showed irregularities High frequency produced smaller contractions. The horizontal bar indicates 100 msec, and the vertical bar 10 g tension.

canals developed maximum tension in the intermediate frequency range.

These patterns of response may be called the utricular type, the lateral canal type and the vertical canal type, respectively

The present study revealed that the activities in the synergistic muscles showed a similar if not the same type of response pattern as in the primary muscles. As an example of the vertical canal type contraction, tension curves of the right inferior oblique (RIO) the right superior rectus (RSR) and the right lateral rectus (RLR) from the stimulation of the left anterior canal nerve (LAC) are shown in Fig. 2. RIO is the primary muscle, RIR, the secondary muscle and RLR, the tertiary muscle of the left anterior canal. As seen in Fig. 2, the modes of their tension curves were similar although RIO the primary muscle showed the largest contractions, RSR the secondary muscle a slightly smaller contraction, and RLR or the tertiary muscle, the smallest contraction.

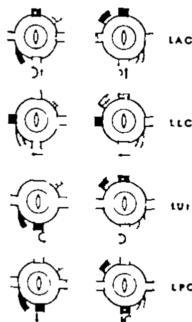


Fig 3 Diagram of the eye movements and muscle activity elicited in the cat by stimulating each vestibular nerve branch. From top to bottom, the linkages between the eye muscles and the left anterior ampullary (*LAC*), the left lateral ampullary (*LLC*), the left utricular (*LU*), and the left posterior ampullary (*LPC*) nerves are presented The primary eye muscles are black, the stronger synergists, cross-hatched, and the weaker synergists, broken oblique line hatched The direction of the evoked eye movements is indicated by arrows.

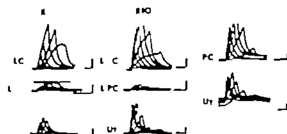


Fig 4 Superimposed eye muscle contraction curves during electric stimulation of single vestibular nerve branches with pulse-trains of constant number of pulses and of different pulse separation.

In the left column (*RLR*), the eye muscle contraction curves of the right lateral rectus are shown. These were induced by stimulation of the lateral canal nerve (*LLC*), the left anterior canal nerve (*LAC*) and the left utricular nerve (*LUt*). RLR is the primary acting muscle of LLC, the tertiary muscle of LAC and LUt as well.

In the middle column (*RIO*), the eye muscle contraction curves of the right inferior oblique are shown. These were induced by stimulation of the left anterior canal nerve (*LAC*), the left posterior canal nerve (*LPC*) and the left utricular nerve (*LUt*). RIO is the primary muscle of LAC, the secondary muscle of LPC and the primary muscle of LUt.

In the right column (*RIR*), the contraction curves of the right inferior rectus muscle are shown. These are induced by stimulation of the left posterior canal nerve (*LPC*) and the left utricular nerve (*LUt*). RIR is the primary muscle of LPC and the secondary muscle of LUt.

Stimulus conditions were similar to Fig. 2. Calibrations are 100 msec and 5 g for each trace.

The primary and the synergistic muscles during stimulation of each of the ampullary and utricular nerves are listed in Fig. 3

Excitation of different single vestibular nerve branches produced different modes of contraction in the same ocular muscle

We have shown that the same extraocular muscle is "innervated" by several vestibular nerve branches. The right lateral rectus (RLR) is the primary muscle of the left lateral canal (LLC) the tertiary muscle of both the left anterior canal (LAC) and the utricle (LUt) (Fig. 4 on the left). As shown in the centre of Fig. 4 the

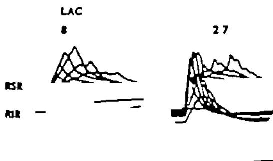


Fig 5 Eye muscle contractions from the anterior canal nerve during electric pulse stimulation of varying intensity Tension increase of the right superior rectus (*RSR*) and the right inferior rectus (*RIR*) induced by stimulating the electrode implanted at the left anterior semicircular canal nerve (*LAC*) with 8 V shown on the left and with 27 V on the right. Pulse trains as used in Fig. were used for stimulation. RSR is the primary muscle of LAC. RIR is the secondary muscle of LUt. Those two muscles are antagonists. The horizontal bar indicates 100 msec, and the vertical bar 10 g tension.

right inferior oblique muscle (RIO) is the primary muscles of the left anterior canal (LAC) and the left utricle (LUt) and is the secondary muscle of the left posterior canal (LPC) the right inferior rectus muscle (RIR) is the primary muscle of the left posterior canal, and the secondary muscle of the left utricle (Fig. 4 on the right)

As seen in Fig. 4 the same muscle re- nded with different modes of contraction ated to the nerve branch stimulated, i.e., the teral canal type, the utricular type or vertical canal type

It may be concluded that each of the vestibular nerve branches preserves its own contraction modes in the eye muscles.

Spread of electric current to adjacent nerves during stimulation of a single vestibular nerve branch manifested in induced eye muscle activities

It was reported that stimulation of a particular vestibular nerve branch activated a particular group of eye muscles and produced the same type of eye muscle contractions when the stimulus remained liminal. When the stimulus intensity was increased beyond threshold, activities were found in other groups of muscles and contraction modes of the extraocular muscles showed changes. In Fig. 5 on the left, contraction curves of the right superior rectus (RSR) and the right inferior rectus (RIR) are shown during stimulation of the left anterior canal nerve (LAC) When stimulated with relatively strong intensity (27 V in this case) both RSR and RIR contracted (Fig. 5 on the right)

RSR and RIR are mutual antagonists. The former is the secondary synergistic muscle of LAC, and the latter the secondary muscle of the left utricle (LUt) Thus, stimulation of LAC through an electrode implanted at the anterior ampullary nerve with relatively weak intensity (8 V in this case) caused contraction of RSR with a vertical canal type response while RIR showed relaxation. The contraction modes of RSR showed changes with stronger stimulation of LAC The magnitude of contraction of RSR was smaller with 27 V stimulation than with 8 V stimulation and the contraction curves showed irregular deformities with 27 V compared with the smooth vertical canal type with 8 V RIR on the other hand showed relaxation with 8 V and contraction with 27 V In addition, the contraction mode of RIR was of the utricular type instead of the vertical canal type. Thus, when stimulating LAC with higher voltages, both RSR and RIR showed contractions, the former with the vertical canal type and the latter with the utricular type. All of these positively indicate that a spread of stimulation occurred and both the anterior ampullary and the utricular nerves were simultaneously stimulated.

COMMENTS AND CONCLUSIONS

In this paper the authors have again shown that each of the single vestibular nerve branches has its own characteristic mode of contraction in any of the three activated eye muscles. Three different modes of contraction are: the lateral canal type the vertical canal type and the utricular type. These relationships

were maintained in each of the three active eye muscles linked to each receptor Since the vestibular labyrinth is small in size and the vestibular nerve branches are located close to each other selective stimulation of single vestibular nerve branches requires a rather skillful and careful technique, and, at the same time, a means for detecting current spread. By utilizing muscle contraction recording, the spread of current to adjacent nerve branches was studied, the results are summarized as follows.

When the vestibular nerve branches on the left side are stimulated, the following indicate the nerve or nerves stimulated.

1 Contraction of the right superior rectus muscle (RSR) indicates anterior canal nerve stimulation.

2. Contraction of the right lateral rectus (RLR) and/or the left medial rectus (LMR) with contraction mode of the lateral canal type indicates lateral canal nerve stimulation.

3 Contraction of the right inferior oblique (RIO) and/or the left superior oblique (LSO) with contraction mode of the utricular type indicates utricular nerve stimulation.

4 Contraction of the left inferior rectus (LIR) indicates posterior canal nerve stimulation.

Each of these is the necessary condition indicating that at least the intended single vestibular nerve branch was stimulated.

If only one of the above-mentioned four conditions appears, it shows that one vestibular nerve branch alone was stimulated. This is both the necessary and sufficient condition indicating that a single vestibular nerve branch was stimulated in isolation.

The spatial separation of the posterior canal nerve from the others is the greatest. Therefore, current spread from an electrode implanted on the posterior canal nerve is expected to be the least likely also spread to the posterior canal nerve from the electrodes implanted on the other nerve branches should be small. This, accordingly makes it possible for us to omit the fourth condition concerning posterior canal stimulation. Namely the necessary and sufficient conditions for indicating single vestibular nerve stimulation for anterior canal nerve are to demonstrate positive (1) and negative both (2) and (3) those for lateral canal nerve stimulation are to demonstrate positive (2) and negative (1) and (3) and those for utricular nerve are to demonstrate positive (3) and negative (1) and (2) To demonstrate positive (4) may be the necessary and sufficent condition for the single posterior canal nerve stimulation.

We have tried to show in this series of experiments why there are different patterns of eye muscle contractions from each vestibular nerve branch when stimulated with pulse-trains. This has proved however to be useful for detecting possible current spread from electrodes implanted at the single vestibular nerve branches.

Those studies which deal with cellular responses in the secondary vestibular neurons and so on during single vestibular nerve branch stimulation, should be carried out to detect minimum current spread (Kasahara et al., 1970) The present method in this paper however can be conveniently utilized for behavioral research.

ZUSAMMENFASSUNG

Die einzelnen Aste des vestibularen Nervs wurden durch die geimpfte Electrode bei der Katze elektrisch gereizt. Verschiedene differente Kombinationen der extraocularen Muskeln wurden von jedem einzelnen, vestibularen Nerv aktiviert. Es war gefunden, dass es drei Typen der von Frequenz-abhängigen Kontraktion der Augenmuskeln gab· d. h., der Typus des lateralen Kanals, der Typus des vertikalen Kanals und der Typus des Utrikulus. Es erwies sich, dass unsere Beobachtung der Kontraktion des Augenmuskels für die Differenzierung benutzt werden kann, ob die vestibularen Nerven entweder einzel oder kombiniert gereizt wurden.

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COCHLEAR NERVE POTENTIALS RECORDED FROM THE EAR CANAL IN MAN

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Abstract Using average technique on an IBM 1800 computer a non-surgical, clinically applicable method of recording action potentials from the cochlea is described. W obtained the components N N₂ and N in response to well-defined transient sounds, which were measured with sound probe. Also, an electro-positive response is described. In response to inverse sound stimulation, different response patterns were found. An H and L-slope in the transfer function of amplitude could be confirmed but in addition we also found kneepoint in the transfer function of latency Simultaneous plotting of standard deviation curves for each recording could be used as an indicator of response and seems to indicate rise in the non-synchronous nerve activity of the background noise. The findings are discussed.

In 1951 Rosenblith & Rosenzweig recorded the intermediate" neuropotentials N and N_2 from the first neurons in the acoustic pathway using electrodes located outside the bulla, and in 1967 *Sohmer & Feinmesser* and *Yoshie* et al. published similar non-surgical recordings in man. It was our aim to develop a system where by the "intermediate" neuropotentials in man could be recorded in a practical clinical way producing distinct recordings of as well N as N_2 and possibly N with low amplitude artifacts and permitting control of the acoustic stimulus.

MATERIAL AND METHODS

Seventeen healthy male and female persons between the age of 4 and 43 were tested, all having normal hearing and normal otoscopy *The persons were placed in the supine position*, and 25 mg largactil was administered i.v to produce a superficial drowsiness after 100 mg mebumal premedication.

Recordings were done in an electrically shielded room with 60 dB sound insulation. The sound was delivered from a TDH 39 telephone specially shielded to minimize electric and magnetic radiation, and consisted of single clicks. Whereas these clicks were produced by electric potentials equal to those shown in Fig. 1 *A* the sound pressure in the ear canal showed a different shape when measured with a sound probe The click had more after-oscillations and appeared with an overshoot opposite the peak (Fig. 1 *B*) The delay produced by the probe *itself* was 0 17 msec determined in the special probe calibration coupler (B & K DB 0260) (Fig. 1 *C*) and in the ear canal the sound had an increased latency of 0.34 msec, measured with the probe i.e. 0 17 msec latency between electric input and sound. All measurements of latency were corrected in accordance with this. Measurements of the sound pressure from the telephone in a three-chamber artificial ear (B & K 4153) (Fig. 1 *D*) showed a nearly identical curve only with a delay of 0 13 msec from electric pulse to the click. Thus it was justified to calibrate the sound pressure from the telephone by means of the three-chamber artificial ear as approximate identity was found

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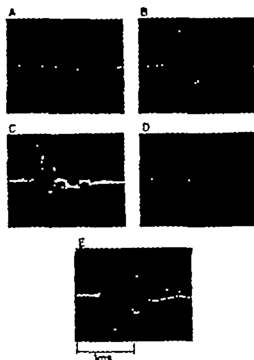


Fig 1 Properties of stimuli. (A) Electric input to telephone (B) Probe measurement of sound pressure in the ear canal. (C) Error introduced by the probe. Comparison between actual sound pressure and probe-measured sound pressure in calibration coupler (B & K DB 0260) (D) Sound pressure in the three-chamber artificial ear (B & K 4153). (E) Comparison between ..d stimuli measured with probe in the ear ..l. Having the same time axis, all recordings begin ..ltaneously with the onset of data collection in the computer Ordinates are arbitrary to facilitate comparison

between the measurements in the artificial ear and the ear canal. Sound pressure was calculated and stated as dB peak equivalent sound pressure level (p.e SPL) i.e dB peak equivalent sound pressure re 0.0002 dyn/cm² Normal persons showed a psycho-acoustic threshold of 25 dB p.e SPL.

On changing the polarity of the electric pulse the sound pressure in the ear canal changed polarity (Fig 1 *E*) Inward movements of the ear drum produced by the peak sound pressure were called condensation (push) stimuli and correspondingly the opposite was called rarefaction (pull)

After local anaesthesia a 0.30 mm enamel insulated and nylon-coated silver wire was passed through a 1 15 mm cannula from planum mastoideum through the back wall of the ear canal After removal of the cannula the chlorided tip, 3 mm long, was fixed under the skin about 2 mm from the limbus and used as active electrode (Fig. 2) A chlorided 0 6 mm silver needle in the ear lobe was used as ground electrode both electrodes were connected to the pre-amplifier with AMP crimp connectors. The electrodes showed a polarization less than 3 mV decreasing to less than 0.5 mV during the session, and had an impedance with a numerical value from 2 kΩ to 6 kΩ within the used frequency range

We built a single-ended 90 dB voltage amplification pre-amplifier with a frequency response from 70 to 5 000 Hz using 10 cm wires. The input impedance was 100 MΩ. Whereas the amplifier gave a 2 $\mu V_{p.p.}$ noise band when shorted with a 5 kΩ resistor the electrodes produced a 4 $\mu V_{p.p.}$ noise band from which the neuropotentials were extracted.

The equipment and intercouplings are shown in Fig. 3 Commanded by the sound delivering gateoscillator (Salomon & Elberling, 1968) an IBM 1 800 computer on line, digitized 300 points with a 16 kHz sampling frequency using 8 bits. The clicks were presented with a time interval of 136 msec in series of 20 pausing 1 sec between series to allow calculation and time

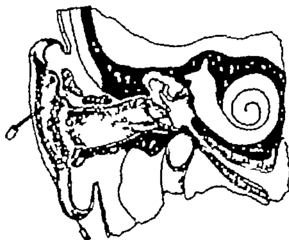


Fig 2 Electrode location, see text.

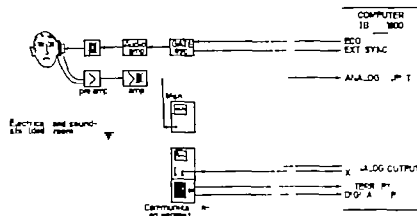


Fig. 3. Block diagram of the equipment used.

sharing. Average calculation was performed using normally 800 stimuli, but according to response amplitude we varied this number from 400 to 2 400 stimuli. In each of the 300 sample points the normally used 800 measurements formed a population. The average curve just mentioned was formed plotting all mean values of every population. A standard deviation curve was plotted from all standard deviations calculated in each population using the formula.

$$\sigma = \sqrt{\frac{\sum_{i=1}^{N} X_i^2}{N} + \bar{X}^2}$$

X = individual measurements
N = total number of stimuli
$\bar{X}$ = mean value

RESULTS

In all 17 consecutive investigated persons a marked N potential was observed, in 16 a clear N and in 12 a just-detectable N was recorded in response to rarefaction stimuli. In all patients a fairly uniform response pattern was found in agreement with Fig. 4. In 8 cases an electropositive component was found superimposed on the response immediately after the trailing edge of the N spike, lasting 2–3 msec.

We found that responses to condensation stimuli differed from responses to rarefaction stimuli. Testing 3 persons the N showed a 0.5 msec and the N_2 a 1.5 msec longer latency in response to condensation stimuli compared with the rarefaction stimuli. Furthermore a somewhat different shape of the response curve was observed (Fig. 5).

In all patients the N was the most stable component, when stimulus intensity was decreased (Fig. 6) and in the later part of the investigation the N_1 could be traced down to the patient's subjective threshold. The N_3 also diminished with decreasing intensities and was only detectable down to 80 dB p.e. SPL, and the N was only seen at intensities of 100 dB p.e. SPL or more.

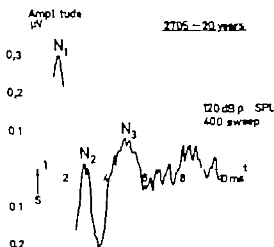


Fig. 4. Typical response. The arrow indicates peak sound pressure in the ear canal.

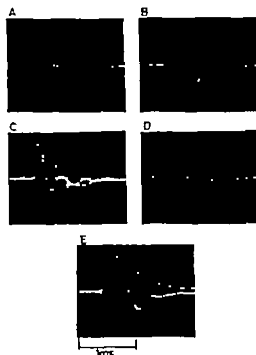


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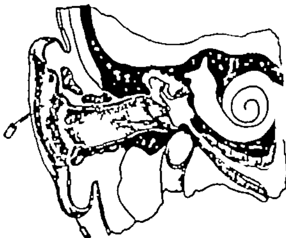


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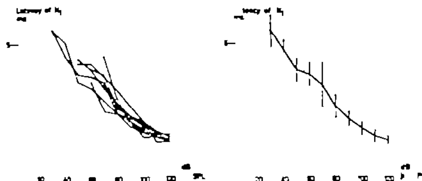


Fig. 7. The latency function of N. The 9 cases with the best resolution of the N peak were used. *Left*, raw data, *right* mean curve with standard deviations indicated.

DISCUSSION

Contrary to earlier authors we felt that only a closed acoustic system would offer well-defined transient acoustic stimulation. A nearly sinusoidal, 2 000 Hz half wave produced a minimum high frequency content and a minimum after oscillation. Our electrode placement did not interfere with a closed system, was simple and, unlike Portmann (1968) and Yoshie & Ohashi (1969), no penetration of the drum took place which possibly introduces acoustic or movement artifacts.

Rosenblith & Rosenzweig (1951) showed that increasing distance between the active electrode and the cochlea decreased the amplitude ratio between N_1 and N_2. Correspondingly Yoshie's investigation (1968) and the present experiments found a ratio of 2 : 1 using a similar electrode placement in the ear canal whereas Sohmer & Feinmesser (1967) demonstrated the ratio 1 : 1 recording from the ear lobe. As Portmann (1968) was right on the promontory his pictures show only a small N_2. As the N_2 and the N_3 components are of diagnostic value as a tool in the retrocochlear tests, an electrode location in the ear canal seems to be the most appropriate.

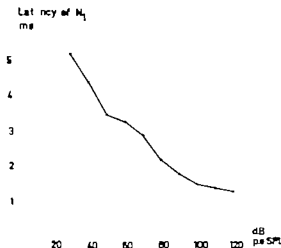


Fig. 8. Mean latency curve for N in three patients, see text.

Our results are in agreement with Yoshie's (1968) regarding the L- and H-function for amplitude versus input intensity but we could also find a broken function in the latency curve for N[1] The kneepoint in our amplitude curve can be calculated about 55 dB SL versus 50 dB SL in Yoshie's paper but the kneepoint for latency was about 45 dB SL and not so well defined. We think that both amplitude and latency curve reflect the double population of elements in the first section of the hearing mechanism.

Like Yoshie (1968) and Portmann (1968) we found an electropositive component in response to clicks. This finding was not constant, possibly in agreement with Rosenblith's (1951) finding, that small extrabullar electrode displacements could change the polarity of cochlear microphonics. The nature of the positive component is still obscure although similarities with summating potentials (Davis et al., 1958) are present.

Although no psycho-acoustic difference between rarefaction and condensation clicks d be observed, both a longer late

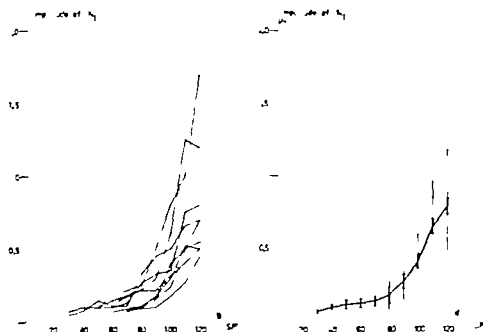


Fig 9 The amplitude function of N The same recordings are used as in fig. 7 *Left* raw data, *right* mean curve with the standard deviation (*thin line*) and the relative standard deviation (*thick line*) relative to 0.1 μV

of response pattern with a relative increase of N latency versus N_1 was observed (Fig. 5) The increase in latency of N_1 is in the same range as the phase shift of sound pressure in the ear canal, when reversing stimulus polarity (Fig. 1 *E*) this raises the possibility that N_1

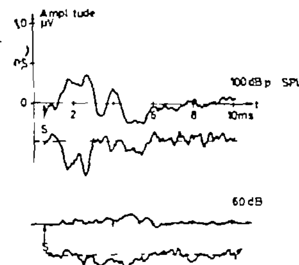


Fig 10 Synchronism between increase in standard deviation and neuro-responses for different stimulus intensities. The standard deviation curves are shown at 1 10 scale

firing is phaselocked to the outward movements of the eardrum. On the other hand, the change of neuro-pattern seems to indicate a different coding of the acoustic neurons due to difference in fluid movements in the cochlea when reversing the stimulus. As no reversion of stimulus seems permissible to eliminate artifacts, no artifacts should after 0.5 msec from the stimulus exceed the averaged background noise On the other hand, comparison of responses to condensation stimuli with responses to rarefaction stimuli seem to offer a possibility of studying the transfer mechanism of the middle ear

The formula

$$\sigma = \sqrt{\frac{\sum_1^N X^2}{N} - \bar{X}^2}$$

can be written as

$$\sigma^2 = \frac{\sum X^2}{N} - \bar{X}^2$$

and

$RMS_Y^2 = RMS_{X+Y}^2 - RMS_X^2$, where:

RMS_X = RMS-value of back ground noise

RMS_{X+Y} = RMS-value of signal and noise

RMS_Y = RMS-value of signal = the calculated mean value.

Thus the σ-curve represents the RMS-value of the background noise. The enlarged value of this curve in the same time area as the neuro-response may indicate that a raise in the activity of the neuro-structures in the vicinity of the cochlea takes place simultaneously with the actual synchronous firing of the first and second neurons.

ZUSAMMENFASSUNG

Wir beschreiben die Anwendung der „average technique" bei einem IBM 1800 Computer, eine zwar nicht kirurgische aber doch klinisch verwendbare Methode, um Aktionspotentiale der Cochlea zu registrieren. Wir haben die Komponenten N_1, N_2 und N_3 als Antwort auf wohldefinierte kurze Lautschälle, die mit einer Messprobe kontrolliert wurden, erhalten. Ausserdem haben wir eine elektropositive Antwort gefunden. Als der Lautschall mit der umgekehrten Polarität erzeugt wurde, wurden verschiedenartige Antwortsmuster gefunden. Wir fanden eine „H" und „L" Neigung bei der Amplitude der Überführungsfunktion die mit den früheren Untersuchungen übereinstimmt. Ausserdem haben wir auch einen Kniepunkt für die Überführungsfunktion der Latenzzeit gefunden. Die gleichzeitige Abbildung von Standarddeviationskurven für jede Aufnahme mit den „average" Kurven war als Indikator der Antwort verwendbar; ausserdem scheint die Standarddeviationskurve eine Erhöhung der nicht synkronisierten Nervenaktivität des Hintergrundsgeräusches anzugeben. Die Resultate werden diskutiert.

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THE INTERAURAL INTENSITY DIFFERENCE AS A DIAGNOSTIC INDICATOR

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Abstract. A simple sound lateralization task, based on interaural intensity differences, has been developed as a tool for diagnosing central auditory disorders. Simultaneous white noise bursts are presented di chotically at an equal sensation level. The intensity is then raised in 1 dB steps in the test ear until the sound image is located at that ear The non-verbal motor response can be carried out by children or by adults with language impairment. Patients who have only peripheral hearing problems respond the same a normal list ners whereas a large percentage of patients with cerebral disease or trauma gi e abnormal results for the ear contralateral to the lesion.

It is well known that when identical sounds are simultaneously presented to the two ears of a listener a fused sound image will be percei ved the midline of the head. Changing the in- ity level of the signal in one ear will result ' the sound image moving toward the ear re ceiving the greater intensity Neurologically impaired listeners frequently do not behave in the predicted manner Atypical lateralization behavior therefore can act as a clinically useful diagnostic indicator

There are a number of tasks based on binaural fusion which have been proposed to test the integrity of the central auditory system. Bocca et al. (1955), Calearo (1957) and others have attempted to evaluate central auditory function by using binaurally presented speech stimuli. On the other hand, Sanchez Longo et al. (1957 1958), as well as other workers, has employed various sound localization tasks for diagnostic purposes. These procedures, how ever are limited in their diagnostic effective ness by certain inherent disadvantages For example, the speech tests are complex to administer and require a verbal response a re sponse many neurologically impaired listeners are not capable of giving. Furthermore there is evidence that neither listeners with peripheral hearing losses, nor listeners with involvement of the central auditory system perform nor mally on sound localization tasks that have thus far been used (Jongkees & Veer 1957 Matzker 1959 Viehweg & Campbell, 1960).

The procedure described below effectively overcomes these difficulties by using simple stimuli. In addition, the response mode is a simple motor task which can be executed by individuals with a wide variety of neurological disorders including various forms of aphasia. Most importantly the task has been shown to differentiate between peripheral and central auditory problems (Pinheiro & Tobin, 1969).

PROCEDURES

Fig. 1 is a schematic representation of the instrumental array used for the sound lateralization task. The output from two noise generators is gated through an electronic switch set for a 10 msec rise-time and an 100 msec duration. The output of the noise generators has a broadband spectrum A two second interval is main tained between signal presentations to avoid

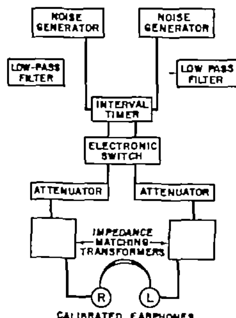


Fig 1 Block diagram of instrumentation.

auditory fatigue. The interval timer is used for this purpose. The noise bursts are delivered dichotically through attenuators and presented to the patient's ears through matched high quality earphones.

Prior to measuring the interaural intensity difference (IID) for lateralization, a threshold of sensitivity for the noise burst signal is obtained for each ear The psychophysical procedure that has been used by the authors is a modified method of serial exploration. However other psychophysical techniques can be used. The only restriction on the technique is that 1 dB increments around threshold are necessary since the normal IID required for sound lateralization is small—on the order of 10 dB (Békésy 1967 Pinheiro & Tobin, 1969)

The IID is defined as the difference in intensity between the dichotic signals that is needed to lateralize the sound image *to the* test ear For this task the dichotic signals are presented at an equal sensation level (SL) A 20 dB SL has been found to be convenient. Intensity is held constant in one ear (the reference ear) and is gradually raised in 1 dB steps in the other ear (the test ear) Lateralization occurs when the sound image is perceived at the test ear The IID score is obtained by subtracting the measured intensity level in the reference ear from the level in the test ear

In the test situation, the patient is instructed to point to the position in his head where the *sound image seems to be localized. It may be* necessary to deliver two or three noise bursts at each intensity step so that the patient can make a judgment about the location of the sound image. After the patient decides on a response, the intensity is increased 1 dB in the test ear This procedure is continued until lateralization is achieved. Both ears are tested in the same manner

With normal listeners the IID should be essentially the same for both ears. The average difference between the IID scores, after having used both ears as references, is about 1 dB with a range from 0 dB to 3 dB. Similar scores are obtained for subjects with sensorineural hearing losses when the above procedures are followed and the ears have been equated for SL. The patient with the peripheral hearing loss should have equivalent IIDs for the two years. When the IID for the two ears is not equivalent, a central auditory system problem may be indicated.

Testing of patients with neurological involvement has revealed that the IID for the ear contralateral to the lesion is abnormally small while the IID for the ipsilateral ear remains within the normal range. The normal IID averages about 10 dB but may range from 7 dB to 13 dB Lateralization to the ear contralateral to the lesion often occurs at equal SL so that the IID for this ear may be 0 dB or close to it. The discrepancy between the two ears may range from 5 dB to as much as 20 dB with an average difference of 8 dB

CLINICAL OBSERVATIONS AND DISCUSSION

Not all patients with clinically demonstrated cerebral disease or trauma will give abn

IID scores for lateralization. About 70% of these patients lateralize sound abnormally (Pinheiro, 1969).

Thus far forty neurological patients have been tested using the procedures described for measuring the IID for sound lateralization. Patients who had abnormal IIDs had neurological signs and symptoms indicating impairment of temporal and/or parietal lobe areas. Patients with lesions limited to the frontal lobe and patients with lesions in the occipital lobe (visual cortex) had normal IID scores.

The exact anatomical and physiological correlates of the abnormally small IID scores in the ear contralateral to cerebral lesions are not known. Neither is it known whether lateralization based on the IID is mediated at the cortical or subcortical levels. It is not yet clear whether the entire auditory system up to some critical point in the system must be intact for normal scores on this task. However the high correlation between cerebral disease or trauma and abnormal IID scores makes the measurement of the IID for sound lateralization a sensitive and useful test for diagnosis of involvement of the central auditory system.

RÉSUMÉ

Un procédé simple de latéralisation du son, basé sur différences d'intensité inter-aurales, a été developpé comme un moyen pour le diagnostique des lésions auditives centrales. On fournit simultanément des eclats de son incolore, mais en separé pour chaque oreille à une intensité de perception égale. En suivant on élève l'intensité du son dans l'oreille examinée chaque fois par un écart de 1 dB, jusqu'à ce que la perception auditive est localisée dans cette même oreille. Cet examin inverbal peut être administré tant aux enfant comme aux adultes ayant la difficulté de parler. Les patients qui offrent des problèmes seulement dans leur audition périphérique donnent les mêmes resultat comme les personnes avec une audition normale tandis qu'un grand pourcentage des personnes avec des maladies ou trauma cerebrales, fournissent des résultat anormales pour l'oreille contre-latérale à la lesion

ZUSAMMENFASSUNG

Es wurde einfache Arbeitsmethode mit Shall Laterisierung für die Diagnose der Horstorungen der inneren Hörorgane erarbeitet, die sich auf die gegenseitigen Unterschiede der Gehörempfindung der beiden Ohren gründet. Man beginnt, indem man gleichzeitig zwar gesonderte, doch gleichwertige kurze neutrale Erregungssignale an die beiden Ohren leitet. Danach wird das Tonsignal an dem untersuchtem Ohr stufenweise um je ein Dezibel soweit gesteigert, bis eine Hörwahrnemung durch dieses Ohr festgestellt wird. Diese wortlose Untersuchung kann sowohl bei Kindern als auch bei Erwachsenen, die Sprachstörungen auf eisen, durchgeführt werden. Bei denjenigen Kranken, die nur peripherische Hörschwierigkeiten aufweisen, ergeben sich dieselben Resultate wie bei normalen Hörern; dagegen weichen bei Hirnkranken oder Gehirntrauma Patienten die Ergebnisse am gegenüber liegenden Ohr vom ersten ab

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PERIPHERAL FACIAL PALSY

Functional Diagnosis

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Abstract A review is given regarding different diagnostic methods in peripheral facial palsy The diagnostic and prognostic value of (1) electromyography (2) electroneurography and (3) electrostimulation is especially discussed. If these tests are used routinely it is possible to perform an early decompression of the nerve in selected cases already within 10–14 days.

Spontaneous peripheral facial palsy (Bell's palsy) is a relatively common affliction among acute cases in a department of otorhinolaryngology or neurology Despite its dramatic onset and the pronounced symptoms, this condition is not a cause for alarm. Spontaneous regression is not uncommon and then usually occurs within a few weeks. There are even authors who believe that this particular form of paralysis always heals spontaneously (Miller 1967) and therefore need not be an object for therapy at all, but experience—at least among otologists—indicates that in certain cases the nerve must be decompressed in its intratemporal course to relieve the strangulation, which is considered to be the most important etiological aspect of the symptoms as a whole. There are difficulties, however in choosing the right cases and indicating the right moment for such surgical intervention. Clinically there is great uncertainty in this respect, so that there is a good reason for making a survey of the most commonly used electro-physiological diagnostic methods, in order to facilitate an early judgement. From the prognostic point of view the type and extent of the palsy is also of great importance.

Diagnostic Methods in Peripheral Facial Palsy

1 Examination of tear production. This can be roughly calculated using the Schirmer-test. Reduced tear-flow is considered to be due to trauma affecting the N intermedius fibres.

2. Examination of saliva production. This is done by catheterizing the salivary ducts, e.g. by sialometry ad modum Diamant (Diamant et al., 1959 Enfors, 1962). Reduced salivary flow is very common when the chorda tympani is included in the palsied area.

3 Examination of the quality of the sense of taste. Especially the salt variation has during recent years been made objective by the development of electrogustometry (Krarup, 1958)

4 Examination of the reflex reaction in the M stapedius. This can also be a valuable aid in diagnosing the level of the palsy The method for such measurements, originally described by Metz (1951), has later been improved by Klockhoff & Anderson (1959), among others.

5 Examination of the neuro-muscular function of the facial nerve is, however the most important test in this connexion, for which the following neurophysiological methods can be used.

IID scores for lateralization. About 70% of these patients lateralize sound abnormally (Pinheiro, 1969).

Thus far forty neurological patients have been tested using the procedures described for measuring the IID for sound lateralization. Patients who had abnormal IIDs had neurological signs and symptoms indicating impair ment of temporal and/or parietal lobe areas. Patients with lesions limited to the frontal lobe and patients with lesions in the occipital lobe (visual cortex) had normal IID scores.

The exact anatomical and physiological cor relates of the abnormally small IID scores in the ear contralateral to cerebral lesions are not known. Neither is it known whether lat eralization based on the IID is mediated at the cortical or subcortical levels. It is not yet clear whether the entire auditory system up to some critical point in the system must be intact for normal scores on this task. However the high correlation between cerebral disease (and abnormal IID scores makes the of the IID for sound lateraliza ve and useful test for diagnosi of the central auditory syste

RESUME

de latéralisation du son, bas d'intensité inter-aurales, a été dev l un moyen pour le diagnostique tives centrales. On fournit simul de son incolore, mais en séparé , e, à une intensité de perception on élève l'intensité du son dans l' chaque fois par un écart de 1 dB jus la perception auditive est localisée dans oreille. Cet examin inverbal peut être tant aux enfants comme aux adultes a; difficulté de parler. Les patients qui offrent des seulement dans leur audition périphérique, les mêmes resultats comme les personnes avec une audition normale, tandis qu'un grand pour centage des personnes avec des maladies ou trauma cerebrales, fournissent des résultats anormales pour l'oreille contre-latérale à la lésion.

ZUSAMMENFASSUNG

Es wurde einfache Arbeitsmethode mit Schall-Laterisierung für die Diagnose der Hörstörungen der inneren Hororgane erarbeitet, die sich auf die gegenseitigen Unterschiede der Gehörempfindung der beiden Ohren gründet. Man beginnt, indem man gleichzeitig zwar gesonderte, doch gleichwertige kurze, neutrale Er regungssignale an die beiden Ohren leitet. Danach wird das Tonsignal an dem untersuchtem Ohr stufenweise um je ein Dezibel soweit gesteigert, bis eine Hörwahrnemung durch dieses Ohr festgestellt wird. Diese wortlose Untersuchung kann sowohl bei Kindern als auch bei Erwachsenen, die Sprachstörungen aufweisen, durchgeführt werden. Bei denjenigen Kranken, die nur periphere Hörschwierigkeiten auf weisen, ergeben sich dieselben Resultate wie bei nor malen Hörern; dagegen weichen bei Hirnkranken oder Gehirntrauma-Patienten die Ergebnisse am gegenüber liegenden Ohr vom ersten ab.

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PERIPHERAL FACIAL PALSY

Functional Diagnosis

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(Received July 17 1970)

Abstract A review is given regarding different diagnostic methods in peripheral facial palsy The diagnostic and prognostic value of (1) electromyography (2) electroneurography and (3) electrostimulation is especially discussed. If these tests are used routinely it is possible to perform an early decompression of the nerve in selected cases already within 10-14 days.

Spontaneous peripheral facial palsy (Bell's palsy) is a relatively common affliction among acute cases in a department of otorhinolaryngology or neurology Despite its dramatic onset and the pronounced symptoms, this condition is not a cause for alarm. Spontaneous regression is not uncommon and then usually occurs within a few weeks. There are even authors who believe that this particular form of paralysis always heals spontaneously (Miller 1967) and therefore need not be an object for therapy at all, but experience—at least among otologists—indicates that in certain cases the nerve must be decompressed in its intratemporal course to relieve the strangulation, which is considered to be the most important etiological aspect of the symptoms as a whole. There are difficulties, however in choosing the right cases and indicating the right moment for such surgical intervention. Clinically there is great uncertainty in this respect, so that there is a good reason for making a survey of the most commonly used electro-physiological diagnostic methods, in order to facilitate an early judgement. From the prognostic point of view the type and extent of the palsy is also of great importance.

Diagnostic Methods in Peripheral Facial Palsy

1 Examination of tear production. This can be roughly calculated using the Schirmer-test. Reduced tear flow is considered to be due to trauma affecting the N intermedius fibres.

2. Examination of saliva production. This is done by catheterizing the salivary ducts, e.g. by sialometry ad modum Diamant (Diamant et al., 1959 Enfors, 1962) Reduced salivary flow is very common when the chorda tympani is included in the palsied area.

3 Examination of the quality of the sense of taste. Especially the salt variation has during recent years been made objective by the development of electrogustometry (Krarup 1958)

4 Examination of the reflex reaction in the M stapedius. This can also be a valuable aid in diagnosing the level of the palsy The method for such measurements, originally described by Metz (1951) has later been improved by Klockhoff & Anderson (1959) among others.

5 Examination of the neuro-muscular function of the facial nerve is, however the most important test in this connexion, for which the following neurophysiological methods can be used.

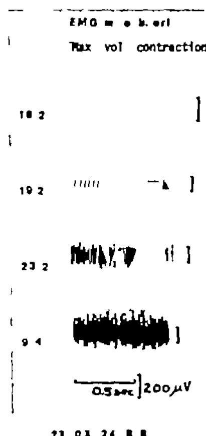


Fig 1 Electromyography Successive regression of Bell's palsy treated with Acton® for months. Electromyographic registration with concentric needle electrodes during ttempted maximum voluntary contraction.

ectromyography

'ith this method muscle action potentials are icked up by intramuscular concentric needle :lectrodes and displayed on a cathode ray tube The muscles are studied at rest, at weak voluntary effort, and at maximum voluntary effort. At rest, the presence of spontaneous fibrillating ictivity suggestive of denervation is noted At slight contraction especial attention is paid to the shape of the voluntary action potentials (e.g. the polyphasic shape of re-innervation potentials)

Increasing voluntary activity during spontaneous regression in a case of Bell's palsy is shown in Fig. 1 Fig. 2 illustrates electromyographic findings after a facial nerve reconstruction performed 36 years after surgical trauma.

Electroneurography

This examination implies electrical stimulation of the nerve trunk via surface electrodes or needle electrodes. The action potentials of the muscle response are picked up by concentric needle electrodes and recorded by an electromyograph. Usually recordings are made from the frontalis muscle as well as from the orbicularis oris and orbicularis oculi muscles. The purpose of this examination is to study the excitability of the nerve and the latency of the muscle response. The amplitude of the electrically induced muscle action potential is seldom suitable for quantitative judgements because of poor reproducibility In order to be certain that really all nerve fibres are stimulated, the stimulation intensity is increased to a level at least 50 per cent above that which is found to give the

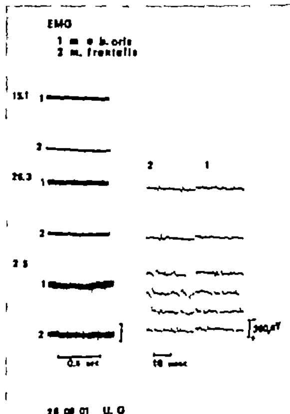


Fig 2 Traumatic facial palsy following an ear operation 36 years previously Neurophysiological restitution during the first 6 months after revision and nerve suture. Continuous (*left*) and sweep recording (*right*) at maximum voluntary effort. Concentric needle electrodes

highest amplitude of the action potential. The findings are compared with the results of the examination on the non-affected side (Fig. 3).

Electrostimulation and strength duration curve

If equipment for electromyographic examination is not available some information may be *obtained from percutaneous stimulation of the* nerve trunk and inspection of the muscles not ing the threshold for minimum contraction. The procedure is performed on both sides. The nerve is stimulated for example at the foramen stylomastoideum using rectangular impulses of 100 msec duration.

Since skin resistance can vary a constant current stimulator (e.g. Siemens "Neuroton") should be used when the threshold of the affected side is compared with that of the non-affected side or when the patient is examined on different occasions.

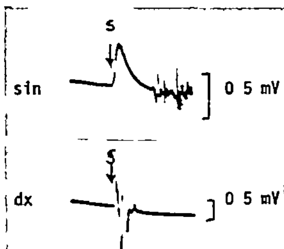


Fig 3 Electroneurography Muscular response to supramaximal stimulation of the facial nerve trunk close to the stylomastoid foramen. The left side (*above*) shows an increased latency of the response as compared with the normal right side (*below*). *S* stimulus artifact. Registration with concentric needle electrodes *from* m. orbic. oris.

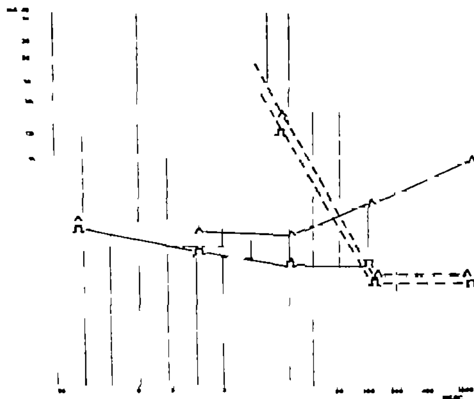


Fig 4 Strength-duration curve ——— normal muscle; — — denervated muscle Rectangular(⎍) and triangular (∧) impulses are used.

In addition, percutaneous stimulation may be performed over the so-called motor points of the muscles noting the threshold at different impulse durations. When rectangular and triangular impulses are used characteristic threshold curves are obtained from normal subjects (Fig. 4) triangular impulses with long duration requiring a higher stimulation intensity due to accommodation of the terminal nerve fibres.

When a denervated muscle is stimulated the strength duration curve rises steeply at the shorter impulse durations. In addition, muscle fibres show no accommodation (Fig. 4)

DISCUSSION

When setting up a programme for extensive clinical routine examinations, it is obviously important to obtain critical evaluation of the different methods from the beginning. Our discussion will therefore primarily be directed to this question. The relevance of the four functional methods of examination named first and their fields of use are best seen from the descriptions of these methods in the quoted literature. Regarding the neuromuscular methods there is on the other hand reason for a closer look at certain results, as there is uncertainty at least among otologists—concerning the ormation that might be obtained and the t occasion for performing the examination. n addition, facial nerve diagnostics touch on *oto-neurology in a wider sense*

The prognostic value of the electrodiagnostic methods can be summarized as follows:

Electromyography

Denervation potentials appear at the earliest two weeks after the injury to the nerve At that time, however it is usually not possible to judge the entire extent of the degeneration Even later denervation potentials can be absent at "routine" examinations, despite the presence of nerve injury

EMG examination *reveals* most cases of clinically complete palsy to be subtotal, that means that single action potentials may be registered at attempted maximum contraction. If action potentials from motor units can be observed in several muscles more than 3 days after the debut of the palsy this is considered a favourable prognostic sign (Granger 1967).

Electroneurography

When there is a total lesion of the facial nerve (e.g. due to cutting of the nerve in certain operations) an electromyographic response may be registered during a period of up to 7 days at maximum electrical stimulation peripherally to the lesion (Gilliatt & Taylor 1959) Similar findings have been demonstrated in cases of Bell's palsy (Taverner 1965) In occasional cases, it has been reported that peripheral excitability has lasted for over 10 days. This appears to be the case especially in zoster oticus. In general, however the prognosis is considered to be good if the nerve trunk is still excitable a week after the palsy has become clinically complete. Now and then one can fail in stimulating the nerve despite the fact that the patient is able to innervate single motor units. This risk is reduced by subcutaneous stimulation with needle electrodes.

In cases of total nerve lesions, the latency of muscle response to stimulation is largely unchanged as long as the nerve is excitable (Gilliatt & Taylor 1959 Taverner 1965) In cases of partial denervation the latency often increases between the 2nd and 4th week (Taverner 1965) *However there is no definite correlation* between the severity of the palsy and the increase of the period of latency

Electrostimulation with inspection of the muscle

Visible muscle response disappears after 3–4 days in cases of total denervation (Gilliatt & Taylor 1959 Etholm, 1967) even if the intensity of the stimulation current is raised to the limit of toleration. Lack of muscle response cannot, however be regarded as a definite sign of total nerve lesion. *The examination should* be supplemented with EMG investigation and

stimulation with needle electrodes. Comparison between the paretic and the non-paretic side with regard to the threshold has also been used for prognostic evaluation (Etholm, 1967).

The signs of denervation appear on the strength duration curve (Fig. 4) only after a couple of weeks following the debut of the palsy. The method thus has no greater interest for early prognostic evaluation.

An operation for decompression is a much debated method of treatment for facial palsy. Most neurologists and some otologists do not consider the operation improves the final results, while a majority of otologists are firmly convinced that decompression is meaningful for a good final result in certain specially chosen cases. When, then, is this intervention to be carried out, and on which patients? These questions have given rise to lively discussions, and there is as yet no ideal method for selecting these cases. Previously the limit was set at 8 weeks and total palsy (Kettel, 1959). This limit was later reduced to 6 weeks and research in the field of electrodiagnostics during recent years has made possible much earlier information as to whether the individual case is due to a physiological block in the nerve or whether there are signs of axon injury, that is, of degeneration of the nerve. In the latter case, decompression ought to be done as soon as possible. Bearing in mind that about 20 per cent of the cases with partial palsy and 60 per cent with total palsy are not restored by conservative therapy (Cawthorne, 1952) and that 20 per cent of those operated on after more than 3 weeks are only partially healed (Lagerholm & Toremalm, 1971) there are very important reasons for considering an *early decompression of the nerve*. It is our experience that this can be done within 10–14 days when an electrodiagnostic follow-up during the initial phase suggests a total lesion with progressive loss of excitability. This opinion is also shared by Jongkees (1969) as well as Hiestand et al. (1969). Modern microsurgical technique entails no noteworthy risks for the patient, but surgical measures must always be based on careful penetration of the situation, and the electrophysiological methods of investigation named in this paper are especially of great value in this connexion.

As a complement to the recently completed 5 year period of clinical investigation (Lagerholm & Toremalm, 1971) a new prospective 5 year series has been begun at the Department of Oto-rhino-laryngology in Malmö in co-operation with the Department of Physical Medicine at the University Hospital in Lund in order to include the electrophysiological tests in the routine examinations.

ZUSAMMENFASSUNG

Übersicht über verschiedene Methoden zur Diagnose der peripheren Gesichtslähmung. Der diagnostische und prognostische Wert (1) der Elektromyographie, (2) der Elektroneurographie und (3) der Elektrostimulation wird besonders erörtert. Wenn diese Untersuchungen routinemässig durchgeführt werden, besteht die Möglichkeit einer frühen Dekompression des Nerven, in ausgewählten Fällen schon innerhalb von 10 bis 14 Tagen.

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THE "RECEPTOR SURFACE" OF THE OLFACTORY ORGAN (EPITHELIUM) OF MAN AND GUINEA PIG

A Descriptive and Experimental Study

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Abstract. A superstructure for the olfactory epithelium is described. The interrelationship of its cellular constituents and their relationship to the free surface of the epithelium is demonstrated. The microvilli in the olfactory region of man are shown to contain an array of filaments, whose pattern of distribution is demonstrated, their mode of branching is discussed. A distinct receptor zone consisting of the terminal processes of the peripheral olfactory neurones forming the outer third of the superstructure of the olfactory epithelium in the experimental mammals is demonstrated. Experimentally induced damage results in swelling and ultimate disintegration of these processes and invariably leads to the disappearance of the receptor zone. There occurs hypertrophy and increase in the number of microvilli f the supporting cells; physio-pathological deformities of their free surfaces are occasionally seen. Later events are recorded and at a week the appearance of the receptor end of the olfactory epithelium is shown

A number of papers have dealt with the ultra structure of the mucosa in the olfactory region (Bloom & Engström, 1952, 1953 Bloom, 1954 Gasser & Palade, 1956 Gasser 1958 De Lorenzo, 1957 1960 Frisch, 1964 1965 1967 Graziadei, 1964 Reese, 1965 Okano, 1965 Andres, 1966 Seifert & Ule, 1967 Okano et al., 1967) Relatively less attention is focused on this region as a whole and on the spatial arrangement of its cellular components which is of importance as the basis of function

Although electron microscopy has added much to our knowledge of the olfactory region in various animals, difficulties are still being encountered. Improved methods in the fixation, embedding and sectioning of olfactory tissue are necessary especially those for the preservation of the seromucinous blanket—a dynamic fluid medium that covers the olfactory epithelium and its surface structures.

Most preparation procedures alter the arrangement and configuration of the cellular processes on the surface of this sensory region and one is faced with the difficulty that until one knows more about the structure of the normal living cell and its behaviour one can only guess whether a given method of preparation preserves it unaltered. Except for gross alterations, judgement as to the value of treatment of the specimen often depends on what the investigator chooses to regard as normal.

The receptor surface of the olfactory epithelium lacks the sterile protective environment of other specialised sensory epithelia. Its surface structures exist precariously Supposedly normal morphological descriptions are often those of olfactory epithelia of laboratory mammals with rhinal infections. These pathological processes cause confusion in the interpretation of the morphological picture

An organised structural pattern for the receptor surface of the olfactory organ (epithelium) in mammals is demonstrated in this paper

In view of the regressive nature

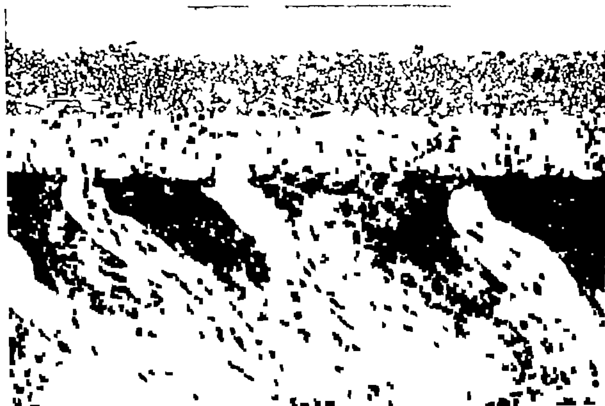


Fig 1 Receptor end of the olfactory epithelium. Coronal section from mid-dorsum endoturbinal I guinea pig showing the relationship of the sensory elements (clear) to that of the supporting cells (dark) and the interrelationship of their processes on the surface Olfactory vesicles (*OV*) lie just beyond the free surface of the epithelium and below the dense peripherally placed zone (*RZ*) of terminal processes (receptor zone). The microvilli of the supporting cells lie in a zone (*MZ*) of their own and surround the olfactory vesicles. The cilium of the olfactory whip tapers to its terminal process in the transitional zone (*TZ*) (see Fig. 3). Electron micrograph 16 000.

y epithelium of man (Naessen, 1970 *b* *e*) d its exposure to atmospheric pollution by chemical substances, drugs and other noxious agents it is important to determine its vulnerability The peripheral olfactory neurones or neurosensory receptors in this study are selectively damaged in the guinea pig and the effects of the experimental lesions in part[1] are demonstrated and discussed.

MATERIAL AND METHODS

The material consists of olfactory tissue obtained in the recent state from 27 guinea pigs, 3 infants, 2 children (17 months and 2 $^1/_2$ years) and 5 human adults (33–51 years)

Fine dissection (embryological) techniques were used to obtain epithelial "shavings and whole thickness "blocks" of mucosa from olfactory tissue immersed in a 3% solution of glutaraldehyde buffered with 0 075 M sodium cacodylate to pH 7.3 (Naessen, 1970 *a*) The specimens were then reimmersed in a 2% osmic acid buffered with Veronal acetate at pH 7.2 and allowed to fix for 2 hours. After dehydration in ethanol, the surface shavings" were carefully mounted flat in glycerine on slides and coverslips applied. They were then examined by phase contrast microscopy The mucosal "blocks were embedded in Epon and sectioned for phase contrast and electron microscopy

[1] Part of a thesis presented at T.C.D Sept. 1968.



Fig 2 Olfactory vesicles. Human adult, 27 years. Tangential section showing marginal ring of origin for the olfactory cilia, it consists of eight basal bodies. Numerous microvilli of adjoining supporting cells surround the vesicles. Electron micrograph 20 000.

OBSERVATIONS

Normal Ultrastructure

Of the various processes of the cells of the olfactory epithelium, most lie superficially at the receptive end of this sense organ. Collectively they form a *superstructure*[1] a great part of which lies or else tapers away beyond the visible range of the light microscope.

The superstructure of the olfactory epithelium consists of olfactory vesicles, sets of olfactory cilia and microvilli (Fig. 1) Whereas these lie submerged in the basal half of the seromucinous blanket (zone of the microvilli) the long distal segments or terminal processes of the olfactory cilia lie individually suspended in its more peripheral layers (receptor zone) close to the ambient air of the external environment.

The *olfactory vesicle* is the terminal end of the peripheral rod-like cytoplasmic extension of the cell body of the olfactory neurone In epithelial "surface shavings the olfactory vesicles as a general rule are observed to lie singly at the interstices and corners of a mosaic formed by the polygonal contour of the free surfaces of the supporting cells (Naessen, 1970 *a*) The vesicles are seen to contain an osmiophilic "marginal ring of basal bodies that give rise to *olfactory cilia* (Fig. 2) The cilia are strongly osmiophilic, and display a radial arrangement. The olfactory cilia in the experimental mammals as observed by the light microscope are but the proximal segments of much longer whip-like structures (or whips[2]) (Fig. 3) whose distal segments or *terminal processes* lie beyond the resolving power of this instrument. The terminal processes are thus the tapering cytoplasmic extensions of the sets of olfactory cilia borne by the olfactory vesicles of olfactory neurones.

The ultrastructure of a terminal process of an olfactory cilium of a guinea pig as seen in cross sections is round or oval and consists of a pair of microtubuli centrally placed in a cytoplasmic matrix enveloped in plasma membrane (Fig. 3 *a*) Occasionally more than two (commonly four) microtubuli are seen within the cytoplasmic extensions of the olfactory cilia. Small circular profiles are sometimes seen incorporated within some terminal processes (Fig. 3 *a*)

The *olfactory cilium* structurally resembles a vibratile cilium as found in the pars respiratoria of the nasal mucosa and trachea (Engström, 1951). Although cilia are structurally alike and common to all parts of the nasal mucosa in man and the experimental mammals their arrangement and relationship to the microvilli on either side of the olfactory margin is different (Figs. 4 5)

Microvilli The free surface of the epithelial cell (supporting cell) in the olfactory region bears atypical microvilli. These may arise singly or in groups from a common stem in structural continuity with an organelle-free homogeneous zone of cytoplasm immediately adjoining the free surface of the cell. This zone of cytoplasm appears to display a degree of plasticity which in turn contributes to the various shapes and sizes that the origins and stems of origin of microvilli consistently show

In counts obtained from tangential sections

[1] A term introduced in this paper

[2] For want of better term.

Fig 5 Receptor surface of the olfactory epithelium. Cross section from mid-dorsum endoturbinal I guinea pig. The surface of supporting cell bearing microvilli is flanked by parts of two olfactory vesicles. Above, numerous cross sections of distal segments of terminal processes form a receptor zone; a few are own longitudinally (*arrow*). An olfactory whip whose terminal process is but the tapering extension of its cilium arises from the side of the olfactory vesicle on the right and lies longitudinally across the field (transitional zone) between a densely packed receptor zone above and a clearer zone of microvilli below (Olfactory vesicle *OV*) Electron micrograph 40 000



Fig 3 Receptor zone of the olfactory epithelium. Guinea pig. The terminal processes collectively display an expansive surface of sensory membrane. Each possesses most frequently and consistently pair of centrally placed microtubules; a few accomodate, in addition, circular profiles (*small arrows*) Occasionally cross sections with four microtubuli are seen (*large arrow*). The terminal processes are singly suspended and loosely held within mucinous mesh of the mucous blanket. Electron micrograph × 60 000.



Fig 4 Receptor surface of the olfactory epithelium. Child, 2 / years. Tangential section cut t the surface through the radial arrangement of the cilia of an olfactory sense cell (olfactory vesicle *OV*) showing their relationship to the microvilli of t least 3 adjoining supporting cells. Electron micrograph 18 000.



Fig 5 Respiratory epithelium. Child, 17 months. Section cut tangentially at the surface showing the arrangement and relationship of cilia to microvilli on the surface of a single epithelial (ciliated) cell. Electron micrograph 35 000.

taken at the surface of the olfactory epithelium in man, an average of 700 microvilli have been estimated to arise singly or from a fewer number of stems of origin per cell. The microvilli possess in their cytoplasm an array of fine filaments. These are longitudinally orientated and circumferentially disposed as seen in the more distal parts of a microvillus (Fig. 6) Proximally however the filaments converge and form a central bundle for the microvilli nearer their origin from the cell surface (Fig. 7)



Fig 6 Receptor surface of the olfactory epithelium. Child, 2 / years. Tangential section showing cross sections f microvilli of the supporting cells. Cross sections of longitudinally oriented filaments are peripherally and circumferentially disposed within the microvilli in thei more distal parts. Arrow shows origin f two microvilli resulting from the bifurcation f common stem. Electron micrograph 96 000.

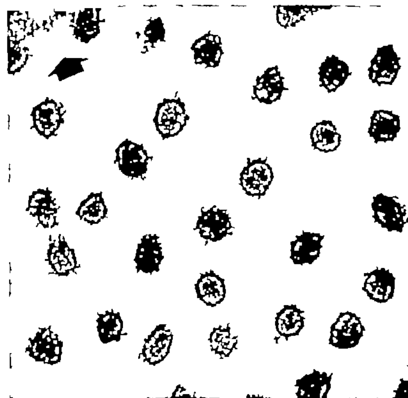


Fig 7 Receptor surface of the olfactory epithelium. Child, 2 ½ years. Cross sections of microvilli nearer their origin from the surface of a supporting cell showing an axial bundle of filaments. Arrow indicates common stem of origin of at least four microvilli. Electron micrograph × 75 000.

Experimental Pathology

The peripheral olfactory neurones were selectively damaged by severing their axons (fila olfactoria) intracranially. Because of its surgical anatomy (Naessen, 1970 *c*) the guinea pig lends itself ideally to this experimental procedure. Animals with rhinitis were excluded from ex (Naessen, 1970 *c*)

Surgical technique

Under Nembutal (Abbott) anaesthesia and with strict asepsis a midline incision was made in the skin on the dorsum of the skull of the guinea pig. The periosteum was incised and its edges were retracted. The animal's head was fixed under an operating microscope. Using a drill, a paramedian coffin lid-like incision was then fashioned on the dorsal aspect of the frontal bone overlying the fossa for the bulbus olfactorius. The lid was gently elevated by means of a hook and removed en bloc.

The dura mater was then incised and the olfactory bulb exposed. Bulb tissue was removed and all intact fila olfactoria at the cribriform plate were severed with a fine knife. By means of focusing and altering the optical plane of the operating microscope the extent of the cribriform plate was defined and the cribriform pattern of its surface was examined for any residual fila that may have escaped section. The wound was then sutured in layers. The animals generally made a rapid and uneventful recovery from the operation.

Experimental procedure

Three series of experiments were conducted each with a batch of 9 guinea pigs (2 of which served as controls) weighing 300–350 g each. An animal in each series was sacrificed at 12, 18, 24, 36, 48, 72 hours and 1 week following operation.

Following decapitation, the ethmo-turbinal complex bone (bearing olfactory epithelium) was rapidly excised and immersed in a 3 % solution of glutaraldehyde buffered with 0.075 M sodium cacodylate to pH 7.3. Specimens for phase contrast and electron microscopy



Fig. 8. Olfactory epithelium. Guinea pig, 24 hours following damage. The distal half of the sensory cell population, staining dark, has degenerated; their peripheral rod-like sensory cell extensions (mainly clear) have withdrawn proximally and appear swollen. Supporting cells are normal, have few or no sensory cell extensions (rods) interposed between them. The mucous blanket at the surface appears ragged (see Fig. 10). Phase contrast micrograph × 470.

were obtained for further treatment as outlined above (see Material and Methods).

Observations

On the damaged side there occurs a selective degeneration of a large proportion of the sensory elements of the olfactory epithelium (Fig. 8). This is accompanied by vital structural changes in the supporting cells. For comparison, a cross section of epithelium obtained from the same site of the olfactory region of another animal[1] (untreated) from the same litter is shown (Fig. 9).

At 24 hours the epithelial changes are striking and the retrograde degeneration appears to attain its maximum in 48–72 hours when most of the sensory cells appeared degenerated. The sensory cell perikarya are shrunk, indented and appear deeply stained; their nuclei are pycnotic. Their peripheral rod-like cytoplasmic extensions retract and show a variable degree of swelling.

Some of the earliest changes following selective damage to the olfactory neurones affect their fine processes and are best seen in the electron microscope at the receptor surface of the olfactory epithelium. The olfactory cilia and their extensions swell to several times their size and then disintegrate (Figs. 10, 11, 12). Within 48 hours the entire receptor zone disappears (Fig. 13). A similar process involves the bundles of unmyelinated axons within the epithelium and in the lamina propria where they constitute the fila olfactoria of the olfactory nerves (*Naessen, 1970 d*).

Olfactory vesicles disappear from the surface as these terminal ends (now devoid of cilia) withdraw between the supporting cells towards the deeper zones of the epithelium for ultimate dissolution. Whereas the disintegration of ol-

[1] Unilateral disease may be present and mitigates against the use of one side as control (Naessen, 1970 c).



Fig. 9. Olfactory epithelium. Guinea pig. Normal. Electron-lucent peripheral sensory cell extensions (rods) are interposed between the darker distal non-nucleated parts of the supporting cells. A broad zone of sensory cell nuclei is conspicuous. An intraepithelial duct of a Bowman gland is showing. The air-surface interface is dense and even, basal half of the mucous blanket is relatively clear (see also Fig. 1). Phase contrast micrograph × 470.

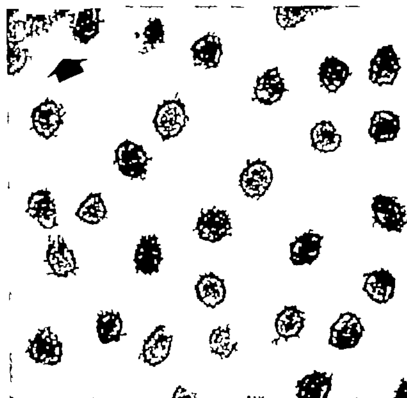


Fig 7 Receptor surface of the olfactory epithelium. Child, 2 / years. Cross sections of microvilli nearer their origin from the surface of a supporting cell showing an axial bundle of filaments. Arrow indicates common stem of origin of at least four microvilli. Electron micrograph 75 000.

Experimental Pathology

The peripheral olfactory neurones were selectively damaged by severing their axons (fila olfactoria) intracranially Because of its surgical anatomy (Naessen, 1970 *c*) the guinea pig lends itself ideally to this experimental procedure. Animals with rhinitis were excluded from ex (Naessen, 1970 *c*)

Surgical technique

Under Nembutal (Abbott) anaesthesia and with strict asepsis a midline incision was made in the skin on the dorsum of the skull of the guinea pig. The periosteum was incised and its edges were retracted The animal's head was fixed under an operating microscope. Using a drill, a paramedian coffin lid-like incision was then fashioned on the dorsal aspect of the frontal bone overlying the fossa for the bulbus olfactorius. The lid was gently elevated by means of a hook and removed en bloc.

The dura mater was then incised and the olfactory bulb exposed. Bulb tissue was removed and all intact fila olfactoria at the cribriform plate were severed with a fine knife By means of focusing and altering the optical plane of the operating microscope the extent of the cribriform plate was defined and the cribriform pattern of its surface was examined for any residual fila that may have escaped section. The wound was then sutured in layers. The animals generally made a rapid and uneventful recovery from the operation.

Experimental procedure

Three series of experiments were conducted each with a batch of 9 guinea pigs (2 of which served as controls) weighing 300–350 g each. An animal in each series was sacrificed at 12, 18, 24 36, 48 72 hours and 1 week following operation.

Following decapitation, the ethmo-turbinal complex bone (bearing olfactory epithelium) was rapidly excised and immersed in a 3% solution of glutaraldehyde buffered with 0 075 M sodium cacodylate to pH 7.3 Specimens for phase contrast and electron microscopy

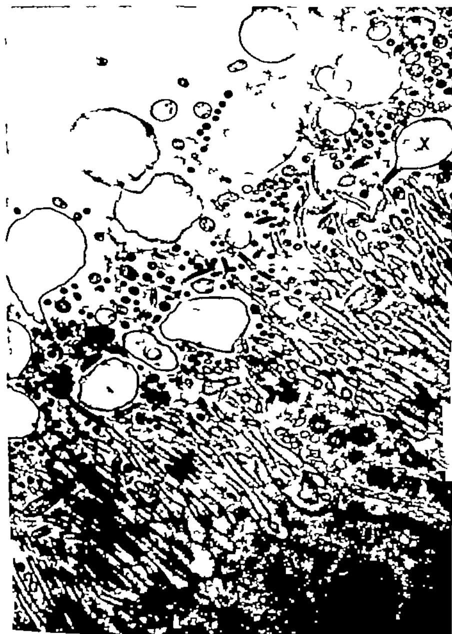


Fig. 11. Superstructure of the olfactory epithelium. Guinea pig, 18 hours following damage. Section shows various stages in the disintegration of the receptor zone (from swelling of the olfactory whips to their ultimate rupture and disappearance). *X* lies within the swollen tip of a terminal process. An olfactory vesicle in retracting from the surface has its basal bodies reoriented and aligned for retrograde course and ultimate dissolution. The increase in numbe and size of the microvilli of the supporting cells is marked. Their mode of branching from hyper trophic common stems of origin is clearly seen. Electron micrograph 15 000.

in freshly fixed "shavings obtained from its surface normally appears smooth (Naessen 1970 *a*). In the experimental state, however the cell's surface presents a characteristic bushy appearance suggestive of cilia. The supporting cells have responded to the specific degeneration of the receptor neurones by hypertrophy and an increase in the number of their micro-



Fig 12 Distal end of the olfactory epithelium. Guinea pig, 36 hours following damage. Section shows an almost total disappearance of the receptor zone of terminal processes. Centrioles (basal bodies) pursue a retrograde course within the withdrawing rod-like extension of a dying neurone (*N*) Increased activity of the supporting cells is shown by (*a*) increased number of mitochondria, (*b*) proliferation and hypertrophy of their microvilli, and (*c*) the frondled appearance of physio-pathological deformities at their distal ends. Electron micrograph 14 000.

villi (Figs. 10 11 12, 13) The stems of origin of the microvilli show marked hypertrophy (Fig. 11) Moreover a variety of deformities at the distal ends of the supporting cells are conspicuous at the surface on occasions (Figs. 10, 12)

At a week the receptor end of the olfactory epithelium is characterised by a conspicuous



Fig. 13. Distal end of the olfactory epithelium. Guinea pig. 72 hours following damage. Section shows the total absence of receptor zone. Continued supporting cell activity is evident. At this phase of the experimental situation there also (see caption 12) occurs hypertrophy and reorientation of the endoplasmatic reticulum (Naessen, 1970 d). Electron micrograph 15 000.

and total absence of its peripheral sensory cell extensions (Fig. 14). Its superstructure consists solely and entirely of a zone of microvilli: these are reduced in number and in size. The cytoplasm adjoining the free surfaces of the supporting cells is less labile and well defined. In other aspects these cells appear normal.

DISCUSSION

The terminal processes of the olfactory cilia do not lie between the microvilli of the supporting cells but lie on a more peripheral plane and form a zone of their own. This is in contradiction to the findings of Okano (1965), An-



Fig. 14. Olfactory epithelium. Guinea pig, 1 week following damage. Section shows the non-nucleated zone and superstructure of the epithelium. There is (*a*) conspicuous absence of electron-lucent sensory cell extensions (rods) normally seen interposed between the supporting cells, and (*b*) absence of the receptor zone. A reduction in the number and size of the microvilli is apparent. The terminal web zone of cytoplasm adjoining the free surface of the cells is well defined; mitochondria reaching it are deflected. The organised appearance and hypertrophy of the endoplasmatic reticulum of the past few days is less conspicuous. Electron micrograph × 17 000.

(1966), Seifert & Ule (1967), Okano et al. (1967) who state or depict in their illustrations an intermixture or intertwine of terminal processes and microvilli. *A similar zone to that* demonstrated here in mammals has been shown by Reese (1965) in amphibia.

No branching of cilia nor a feltwork of streamers as described by Gasser & Palade (1956) in the pig have been seen in the guinea pig.

Because of their position, structural differentiation and expansive surface it is not unreasonable to assume that the terminal olfactory processes represent the true sensory receptors subserving the sense of smell. The terminal processes may well be regarded as *dendrites* and the membrane enveloping these processes be collectively regarded as the "receptor membrane" of the olfactory neurone. It would then *follow that the bipolar olfactory neurone* of light microscopy is in fact a multipolar cell.

The olfactory area in man lies at the apex of a system of gutters or nasal watershed (Naessen, 1970) and the effective propulsion towards choanae and nares of an extensive mucous sheet exerts a significant drag on the seromucinous blanket covering a restricted olfactory area and consequently helps in the drainage of its glandular secretions. It is likely that such a drag would help align the terminal processes of the olfactory cilia in the direction of the respiratory region (*Figs. 3, 3 a*). The ol-

factory cilia may provide a counterforce to prevent their extensions (terminal processes) from being carried away with the stream of mucus and thus help to retain them in their proper anatomical position within the receptor zone of the olfactory region. They may do this by displaying a whip-like action or tag force acting from below movements related to their structure as kinocilia.

Whereas the nucleated part of the peripheral olfactory neurone enjoys relative protection in the sealed off deeper layers of the epithelium the major part of its cytoplasm lies in a peripheral extension whose terminal end (olfactory vesicle) at the surface gives rise to a simple yet extensive receptor mechanism The receptor zone of the olfactory epithelium would thus appear to be most vulnerable. There is evidence to suggest that if this be damaged by inflammatory processes, drugs and volatile noxious agents the supporting cells in the olfactory region respond by hypertrophy and proliferation of their microvilli or by the development of cilia (Naessen, 1970 *a*, *d*) Surface shavings examined microscopically reveal a bushy appearance of an altered epithelial surface consistent with either Differentiation is difficult and can only be solved by the examination of sections by phase contrast microscopy or at levels of resolution obtained in the electron microscope. Microvilli generally arise from common stems and possess no basal bodies their arrangement is much less ordered than that of cilia. In damaged states of the olfactory epithelium the early disintegration and disappearance of its receptor zone demonstrated above exposes the underlying zone of altered microvilli and displays clearly the bushy appearance they present.

Following sensori-neural damage the supporting cell in the olfactory region is shown in this study to display great activity The potential degree of plasticity of its cytoplasm is reflected in the cell's ability to give rise to common stems of origin of varying sizes for its proliferating and greatly hypertrophied microvilli (Fig. 11) and to a bizarre variety of other deformities at its distal end (Figs. 10, 12) In a week the supporting cells show signs of regression in their activity of the past few days ultrastructural appearances at the surface suggest a return to normal"

No bladder-like structures or bulbous swellings (terminal or otherwise) have been seen associated with the terminal processes of the peripheral olfactory neurones. These artefacts have in the past been considered ultrastructural entities with possible functional significance They are common in mammals with rhinal disease and have been reproduced here by induced damage' their ultimate fate is clearly demonstrated. Occasionally small circular profiles are seen incorporated in the cytoplasmic matrix of a terminal process (Fig. 3 *a*) These may represent an early degenerative change.

Following experimentally induced or spontaneous damage basal bodies aligned in rows have been observed in sets within the rod-like extensions of the peripheral neurones at various levels of the olfactory epithelium. An apparently similar picture arises in the developmental stages of these neurones and unless viewed in association with other signs of degeneration of the epithelium as a whole dying neurones are liable to be mistaken for developing neurones or regeneration of olfactory cilia.

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ZUSAMMENFASSUNG

Eine obere Struktur des olfaktorischen Epithels wird beschrieben. Die Beziehung ihrer Zellenbestandteile und deren Relation zur freien Oberfläche des Epithels wird gezeigt.

Es wird gezeigt, dass die Mikrovilli in der olfaktorischen Region beim Menschen eine Anordnung von Fasern enthalten, deren Distributionsmuster demonstriert wird. Ihre Art, sich zu verzweigen wird besprochen. Eine bestimmte Receptorzone, die aus den endgültigen Prozessen der peripheren olfaktorischen Neuronen besteht, und das äussere Drittel der oberen Struktur des Olfaktorepithels in Versuchstieren

bildet, wird bezeigt. Experimentell verursachte Zerstörung resultiert in Schwellungen und ultimater Desintegration dieser Prozesse und führt unweigerlich zum Verschwinden der Receptorrone. Dort entsteht Hypertrophie und ein Zunehmen der Anzahl Mikrovilli von den unterstützenden Zellen. Physisch–pathologische Deformierungen ihrer freien Oberflächen kommen gelegentlich vor Spätere Erscheinungen wurden beobachtet, und innerhalb einer Woche kann das Erscheinen des Receptorendes des olfaktorischen Epithels gezeigt werden.

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THE EFFECT OF IRRADIATION IN HIGH DOSES ON PAROTID GLANDS

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Abstract. On radiation therapy of malignant tumours in the head and neck region, the salivary glands are often situated in the radiation field. Ten patients undergoing radiation therapy with high doses for tumours in the region of nasopharynx, lingua and bucca have been examined with respect to the function of the parotid glands. The glands received doses of 4 400–7 200 rad, and the function has been investigated by sialographic and sialometric methods. The sialograms show that one year or more after irradiation the parotid glands have significantly smaller projected area than the non-irradiated glands. The irradiated glands had a sparser duct system than the non-irradiated ones. The sialometric examinations showed that all the irradiated glands stimulated with 1% citric acid produced very little saliva. The secretion was in all cases remarkably lower than in a normal material, and in most cases only few drops were collected during 10 min. In spite of marked decrease in secretion at the end f the irradiation treatment the size of the glands was still unchanged. Only a certain time after irradiation was the decrease in size observed. The reason for this is discussed.

When radiation therapy is applied to malignant tumours, the adjacent normal tissues are more or less damaged. High doses (6 000–7 000 rad) are mainly given where the tumour is so located that total extirpation by surgical operation would be difficult to realize. In the head and neck region several such types of tumour occur such as malignant lymphoma and low differentiated carcinoma located in the region of the tonsils and the nasopharynx. Furthermore, preoperative irradiation with doses of 4 000–4 400 rad is often used on some other tumours in the head and neck region, e.g. cancer of the tongue, gingiva and bucca. When radiation therapy is applied to tumours in the head and neck region acute as well as chronic changes appear in the mucous membrane of the nose, mouth and throat. However not only the mucous membrane and the minor intraoral salivary glands are damaged, but in many cases also the major salivary glands, i.e. the parotid glands, the submandibular glands and the major sublingual glands—if the glands are situated within the therapy beam. This is why radiation therapy of head and neck tumours often causes a disturbance of the function of the salivary glands, with consequent dryness of the mouth and throat. Furthermore, these patients show increased frequency of caries.

The purpose of this investigation is to study how the function of the parotid glands is influenced by irradiation. The critical dose level for disturbances of the function and the reversibility will be discussed in subsequent papers.

The function of the parotid glands has been investigated with sialometric and sialographic methods.

MATERIAL

The material consists of 10 patients undergoing radiation therapy for tumours in the region of the head and neck. Five of the patients were

Table I. *Five individuals receiving high-dose radiation therapy and examined 1 year or more after the period of the radiation therapy (group I)*

		Age at time of			Dose in rad to parotid glands	
No.	Sex	Treatment	Examination	Diagnosis	Left	Right
1		26	35	Carcinoma linguae	6 500	6 500
2	♀	45	47	Nasopharyngeal carcinoma	4 400	4 400
3	♀	45	46	Nasopharyngeal carcinoma	6 500	6 500
4	♂	50	51	Nasopharyngeal carcinoma	6 600	6 600
5	o	60	62	Carcinoma linguae sin.	6 500	650

examined with sialometric and sialographic techniques one year or more after radiation therapy (group I). The remaining five patients (group II) were examined with sialographic technique just before radiation therapy and two of these also just at the conclusion of the therapy. Three of the five patients in group II were examined with sialometry just before the radiation therapy and all five just at the end of the radiotherapeutic period. The sex and age at the time of treatment and on examination, the diagnoses of the tumours and the doses given to the parotid glands are given in Table I (group I) and in Table II (group II). All patients were irradiated externally. Eight patients were treated with the Siemens I cobolt 60 unit, giving a dose rate of 40–60 rad/min, while two patients were treated with the Varian linear accelerator operating at 6 MV and at 200–250 rad/min. The parotid gland dose was calculated from the physical dose planning carried out for each patient. All patients received fractioned therapy. Treatments were performed with a tumour dose of 100 to 250 rad a day. A total dose of 4 400 to 7 200 rad was given to the parotid glands for a period of six to eight weeks. Cases 5 and 7 got only a smaller dose of approximately 650 rad to the right parotid gland (Tables I and II).

METHODS

Sialography is the roentgenologic demonstration of the ductal system of the salivary glands after the injection of contrast medium into excretory ducts. A blunt, pliable silver cannula connected with a polyethylene tube is introduced into the duct. The injection of the contrast medium is given manually at a slow rate from a syringe. Three sialograms are exposed: the first one when the patient signals that he feels a sense of tension, the second and third when he signals a light and a moderate pain, re

Table II. *Five individuals with high-dose radiation therapy and examined before and immediately after the period of radiation therapy*

				Dose in rad to parotid glands	
No	Sex	Age at time of treatment and examination	Diagnosis	Left	Right
6	♂	45	Nasopharyngeal carcinoma	6 650	7 200
7	♂	47	Carcinoma buccae sin.	6 500	650
8	♂	53	Nasopharyngeal carcinoma	6 150	6 150
9	♂	66	Nasopharyngeal carcinoma	6 400	6 450
10	♂	71	Nasopharyngeal carcinoma	6 400	6 500

Table III. *The area of the parotid glands of the individuals in group I and group II*
Figures in brackets are the area at the end of irradiation period

Group I				Group II		
Pat. no.	Interval between treatment and examination in years	Area of parotid glands in cm² Left	Right	Pat. no.	Area of parotid glands in cm² Left	Right
1	9	6.8	7.2	6	14.0 (13.8)	11.2 (12.0)
2	2	6.5	9.0	7	16.6	14.5
3	1	9.6	10.1	8	19.0	14.3
4	1	11.1	11.8	9	19.0 (19.4)	19.5 (19.6)
5	2	8.4	(14.5)	10	24.8	18.5
Mean values and S.D of irradiated glands		8.9±1.9			17.1±3.9	

spectively (Ericson, 1968). The contrast medium used was 60% Urografin.

In the assessment of the sialograms there are two main factors of interest: the appearance and frequency of the ducts and the projected areas of the parotid gland. The area of the gland in the sialograms can be planimetrically calculated with a high degree of precision and reproducibility and moderate deviations in the orientation of the object are of no significance when compared to the intersubject variation in the size of the gland (Ericson, 1970). The area was measured on two different occasions and on two different sialograms of each case. The measurements were performed with a planimeter, Ingnt 9544-11 type OTT.

The sialometric method is a modification of the method described by Enfors (1962) and Ericson (1968). During the secretory determinations the patient reclined in a modern dental chair with the sagittal plane parallel with the vertical plane. The recordings were made during rest and during stimulation with citric acid. One per cent solution was used for the stimulation of the glands. The rate was 3 drops every 30 sec for 10 min. The citric acid was applied in the midline of the anterior third of the dorsum of the tongue. The patient was instructed to move his tongue after every instillation and to swallow between the instillations. The stronger stimulation by 6% citric acid could not be used, as the intraoral tissues were affected by the irradiation and the patients complained about the smarting pain.

The saliva was collected with a combined suction and collecting plastic cup over the orifice of Stenson's duct. The collecting cup was connected with a small plastic container by means of a polyethylene tube. The recordings were made simultaneously on the left and right sides during 10-min periods. Before and after the recordings the collecting cup, polyethylene tube and plastic container were weighed while enclosed in a plastic box. It may be noted that one drop of saliva weighs approximately 0.050 g according to the recording technique described by Enfors (1962) and approximately 0.060 g according to Ericson (1968). The weighing procedure is a very simple method for recording the amount of saliva. The accuracy is very high in the weighing procedure used and is more than 100 times higher than the weight of one drop of saliva.

As is pointed out by Ericson (1968), errors due to the apparatus, deviations in application and intraindividual variation are of subordinated importance as compared with the interindividual variation of the subjects. The best intraindividual correlation is obtained in connection with the citric acid stimulated secretion (Ericson, 1969). In our material it was



Fig 1 Sialogram of a parotid gland obtained 9 years after the radiation therapy (Case 1). Natural size

found that the stimulation effect was observable on some occasions only after 1 to 2 min. The 10-min period for collecting the saliva will therefore further diminish the intraindividual errors.

RESULTS

Sialography

The individual values for the areas of the parotid glands are tabulated in Table III. All values of the single parotid glands are means of two separate determinations on the two different sialograms best filled by contrast medium. In



Fig 2. Sialogram of a normal parotid gland before radiation therapy (Case 9). Natural size.



Fig 3 Sialogram of the same case as in Fig. 2 at the end of the radiation therapy Natural size.

group I, comprising the patients 1–5 the sialographic examination was carried out one to nine years after the radiation therapy was completed. The right parotid gland of patient 5 was outside the radiation field during the therapy and has been excluded from the calculations. The area of the remaining nine glands varied between 6.5 and 11 8 cm^2 with a mean value of 8 9 $\pm$ 1 9 cm^2

The area of the right parotid gland of patient 5 who got a small radiation dose, was 14.5 cm^2 which is more than the area of any of the other irradiated glands in group I.

In group II the radiographic examination was performed just before the radiotherapy of patients 6–10 and in cases 6 and 9 also just after the radiation therapy was completed. The areas of the ten parotid glands measured before irradiation varied between 11.2 cm^2 and 24 8 cm^2 with a mean of 17 1 $\pm$ 3 9 cm^2

In the two cases in which the area of the parotid glands was measured before as well as just at the end of the treatment there was no difference in area.

A comparison of the mean values for the areas of the parotid glands in the two groups showed that those of group I were significantly smaller than those of group II (0 001 $< p <$

Table IV *Resting and 1% citric-acid stimulated secretion of the parotid glands of the individuals in group I*

Pat. no.	Interval between treatment and examination in years	Mean values for resting secretion of irradiated parotid glands (Mg/10 min) (9 glands)	Mean values for stimulated secretion (1% citric-acid) of irradiated parotid glands (Mg/10 min) (9 glands)
1	9	13	1 840
2	2	17	419
3	1	3	5
4	1	20	51
5	2	14	18
Total and S.D		13 ± 6	467 ± 787

0.01) i.e. glands irradiated one year and more before the sialography were significantly smaller than nonirradiated glands and glands examined just at the end of the radiation therapy.

In group I all sialograms, except that of case 5 right parotid gland, which got a small dose, were characterized by a sparse duct system (Fig. 1). In case 2 left parotid gland, the main duct was dilated and showed stricture formations. In group II the sialograms showed normal parotid glands (Figs. 2, 3) with the exception of those of case 6. In that case the sialograms showed globular spherical accumulations of contrast medium, indicating sialodochiectasies.

Errors of the method

The precision of the planimetric measurements was determined with the help of the double determinations. The mean difference between the values from the two measurement occasions was 0.1 cm² indicating that there was no systematic difference between the two occasions. The standard deviation for a single determination was 0.23 cm². Thus the error of measurement is small compared to the great variation between the individual glands (Table III).

Sialometry

The individual values for resting secretion and secretion on 1 per cent citric-acid stimulation are given in Tables IV and V.

The secretion values are tabulated as mean values for the two parotid glands of each patient in those cases where both glands have got high doses and the left glands of the two cases (patients 5 and 7) receiving unilateral

Table V *Resting and 1% citric-acid stimulated secretion of the parotid glands of the individuals in group II. Values before (3 cases) and at the end of the irradiation period (5 cases).*

Pat. no.	Mean values for resting secretion (Mg/10 min)		Mean value for stimulated secretion (1% citric acid) (Mg/10 min)	
	Before irradiation (6 glands)	At end of irradiation (9 glands)	Before irradiation (6 glands)	At end of irradiation (9 glands)
6	17	5	1 235	10
7	—	77	—	70
8	78	26	2 736	34
9	135	33	4 378	280
10	—	39	—	197
Total and S.D	77 ± 59	36 ± 26	2 783 ± 1 572	118 ± 115

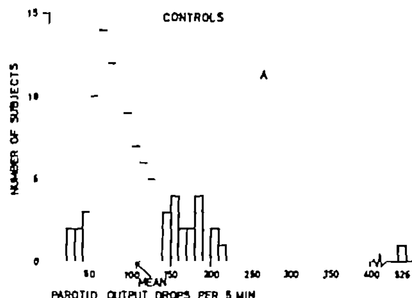


Fig 4 One per cent citric-acid stimulated secretion of parotid glands in a "normal" population (92 individuals). The secretion is expressed as the output, in drops of left plus right sides during 5 min, which corresponds to the mean output of each gland during 10 min (1 drop weighs 60 mg). From Ericson, 1968.

radiation therapy As has been mentioned before the right parotid glands of two cases (patients 5 and 7) received only secondary radiation, approximately 650 rad. The two glands mentioned have for that reason been excluded from the calculations of mean values and S.D shown in Tables IV and V The interindividual variations in the results of the sialometric examinations are great. However notwithstanding this variation, the effect of the irradiation of the parotid glands is clear at any rate with reference to the stimulated secretion. The rest secretion of parotid glands irradiated with doses is very low In all cases the mean e for the secretion of the two glands is less than 77 mg (1 drop weighs 60 mg) during a period of 10 min. Comparison of the resting secretion before and after irradiation in group II shows that the secretion decreases in all cases. The secretion on stimulation with 1 per cent citric acid is, after irradiation, less than 419 mg per 10 min, except for patient 1 i.e. 8 drops or less as a mean for the glands. This result may be compared with the secretion values obtained from a control material of 92 individuals, 26–64 years old, with clinically and roentgenologically normal parotid glands (Ericson, 1968) The mean value for the secretion of the normal parotid glands on 1 % citric acid stimulation was 6 300 ± 3 981 mg per 1 min (Fig. 4) In no case was the secretion le than 1 200 mg per 10 min. Thus, the irradi tion given to the parotid glands results in very marked decrease of the secretory capaci in all cases except one. The latter (patient however has a value which is represented that part of the normal material having th lowest secretion value (Table IV) Patient 1 the youngest, and had been treated for as lo as 9 years before the examinations (Table I).

In two cases, patients 5 and 7 it is possib to make an intraindividual comparison b tween the reaction of a parotid gland whic received a high dose and a gland affected onl by a low dose, or approximately 1/10 of th high dose. No marked difference is noticed regards the resting secretion. However th values for the stimulated secretion of the strong ly irradiated glands decrease to a great exten The ratios of the stimulated secretion of th low-dose irradiated (650 rad) and high-dos irradiated glands (6 500 rad) of patients 7 an 7 were 626 18 mg per 10 min and 1 000 7 mg per 10 min respectively

DISCUSSION

The most common secondary effects of radia tion therapy applied to tumours in the region o

the head and neck are a decrease in the amount of saliva, with consequent dryness of the mouth (xerostomia) and an increased frequency of caries.

In the present study the effect of irradiation with high doses on the function of the parotid glands has been investigated. The irradiation effect on the anatomy has been examined by sialography to study the ductal system and the size of the glands. A marked change of the ductal system seems to appear a certain time after the irradiation, as the four glands examined just at the end of the period of radiation therapy showed, a sialogram similar to those obtained before the therapy while all glands examined one year and more after the irradiation were characterized by a sparse duct system. As regards the size of the parotid gland it has been shown by Ericson (1970) that the area of the gland projected on a sialogram is strongly correlated to its volume. For calculation of the volume from the value for the area the following formula is given: Volume (in cm^3) $= -13.44 + 2.32\,x$, where $x =$ area in cm^2. For small glands this formula might be inadequate. The area has for this reason been used as the criterion of the gland size.

Table III shows that the four glands (cases 6 and 9) measured at the beginning and end of the period of irradiation do not decrease in size. On the other hand, a comparison of the size of the glands examined just at the termination of the irradiation with that of the glands examined one year and more after radiation therapy shows a marked decrease in size. The histological changes in the tissues of salivary glands on irradiation may give an explanation for the observations on the structure of the duct system as well as the size of the gland. Evans & Ackerman (1954), Kashima et al. (1965) and Wallenborn et al. (1969) have shown that irradiation of the parenchyma first of all damages the serous alveoli (acini) while the duct structures are not affected. During the irradiation the number of serous alveoli diminishes. Owing to oedema the interlobular connective tissue becomes swollen. After the irradiation the oedema decreases, while the stroma fibrosis and sclerosis progress. The effect of irradiation on histology may explain the anatomical changes studied on the sialogram. The decrease in size is seen only a certain time after the irradiation. This may be due to the fact that the reduction of the acini caused by the irradiation is concealed by a compensatory swelling of the stroma. After the irradiation the exudation disappears and the proliferative fibrosis develops successively while the parotid gland begins to decrease in size. As a consequence of the diminished number of functioning secretion cells the duct system seems gradually to disappear. In the nine glands examined one year or more after radiation therapy the mean of the sizes of the parotid glands (group I) was significantly smaller than the mean of the sizes of the ten glands in group II before irradiation.

According to Ericson (1968) there is a high correlation between the size and secretion value of the parotid gland. In the present study a marked exception to this rule is seen. The four glands of patients 6 and 9 were examined by sialometry and sialography before the radiation therapy as well as just at the end of the period of irradiation. In spite of a marked decrease of the secretion the size is unchanged at the end of the irradiation period (Tables III, V). The irradiation effect on serous alveoli may cause the decrease in the secretion, while the decrease in the size may be observable only a certain time after the irradiation, probably only after the disappearance of the exudation and oedema and the successive diminution of the duct system. Consequently a sialometric examination is very valuable for an estimation of the function of the parotid gland—the area of the gland projected on a sialogram might be misleading for an estimation of the gland function just at the end of the radiation therapy.

All the patients except one were 45 years or older at treatment. The exception was 26 years old at the time of the radiation therapy and was examined for as long as 9 years after the irradiation. This patient has a markedly

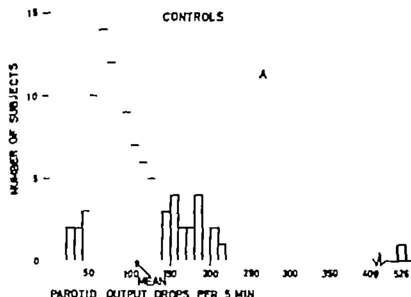


Fig 4 One per cent citric-acid stimulated secretion of parotid glands in a "normal" population (9 individuals). The secretion is expressed as the output, in drops, of left plus right sides during 5 min, which corresponds to the mean output of each gland during 10 min (1 drop weighs 60 mg). From Ericson, 1968.

radiation therapy As has been mentioned before, the right parotid glands of two cases (patients 5 and 7) received only secondary radiation, approximately 650 rad. The two glands mentioned have for that reason been excluded from the calculations of mean values and S.D shown in Tables IV and V The interindividual variations in the results of the sialometric examinations are great. However notwithstanding this variation, the effect of the irradiation of the parotid glands is clear at any rate with reference to the stimulated secretion. The rest secretion of parotid glands irradiated with h doses is very low In all cases the mean for the secretion of the two glands is less than 77 mg (1 drop weighs 60 mg) during a period of 10 min. Comparison of the resting secretion before and after irradiation in group II shows that the secretion decreases in all cases. The secretion on stimulation with 1 per cent citric acid is, after irradiation, less than 419 mg per 10 min, except for patient 1 i.e. 8 drops or less as a mean for the glands This result may be compared with the secretion values obtained from a control material of 92 individuals, 26–64 years old, with clinically and roentgenologically normal parotid glands (Ericson, 1968) The mean value for the secretion of the normal parotid glands on 1 % citric acid stimulation was 6 300 ± 3 981 mg per 10 min (Fig. 4) In no case was the secretion less than 1 200 mg per 10 min. Thus, the irradiation given to the parotid glands results in a very marked decrease of the secretory capacity in all cases except one. The latter (patient 1) however, has a value which is represented in that part of the normal material having the lowest secretion value (Table IV) Patient 1 is the youngest, and had been treated for as long as 9 years before the examinations (Table I).

In two cases, patients 5 and 7 it is possible to make an intraindividual comparison between the reaction of a parotid gland which received a high dose and a gland affected only by a low dose, or approximately 1/10 of the high dose. No marked difference is noticed as regards the resting secretion. However the values for the stimulated secretion of the strongly irradiated glands decrease to a great extent. The ratios of the stimulated secretion of the low-dose irradiated (650 rad) and high-dose irradiated glands (6 500 rad) of patients 7 and 7 were 626 18 mg per 10 min and 1 000 70 mg per 10 min respectively

DISCUSSION

The most common secondary effects of radiation therapy applied to tumours in the region of

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A COMPARISON OF THE IMMUNE RESPONSE OF TONSILS WITH THE APPENDIX AND SPLEEN IN NEONATAL RABBITS

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(Received July 28, 1970)

Abstract Utilizing the local hemolysis-in-gel test, it was found that rabbit tonsils could respond to tetanus toxoid when the animal was injected at 1 14 days of age and challenged 5–10 days later In contrast with the appendix and spleen which gained immunological competence, the tonsils decreased in plaque-forming ability during the early neonatal period. The 10 day challenge period consistently proved to elicit stronger immune responses than did the 5 day period in all three organs throughout the 6 week period. The palatine mucosa provided successful antigenic route for tonsilar stimulation.

The fact that the lymphoid structures, the tonsils, occupy a strategic position in the nasopharynx, and the fact that they parallel the thymus with respect to time and place of embryonic origin and like the thymus, involute with the approach of puberty (Barnes, 1923) suggest that they might be implicated in the development of the immunological system. We have attempted to investigate the immunological characteristics of the tonsils of rabbits during the first 6 weeks after birth. The immune response obtained in tonsils was compared with that of other rabbit lymphoid organs of the same age. The effects on tonsilar immune response of the number of days allowed to elapse after primary injection and the route of antigen introduction were also tested. Because Immunologlobulin M (IgM) appears to be the predominant antibody in the initial primary immune response (Uhr et al. 1962) as well as the first antibody to be synthesized embryologically (Bellanti et al., 1962, 1963 Uhr & Finkelstein, 1963), a modification of the local hemolysis in gel (LHG) test (Jerne & Nordin, 1963) which primarily detects this large immunoglobin, was used.

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MATERIALS AND METHODS

Animals

New Zealand white rabbits from ages 18 hours to 52 days were used. Does and/or their weaned offspring were fed a standard ration of rabbit food pellets (Big Red rabbit food pellets, Country Best, Agway Syracuse, N Y) Only those animals whose sera was found to give a negative Ouchterlony test (Ouchterlony 1953) for precipitins to tetanus toxoid prior to immunization were used.

Age

Rabbits were injected at ages ranging from less than 24 hours *post natum* to 42 days of age with challenge intervals of 5 and 10 days.

Antigens

Tetanus toxoid, provided for this study by Mr William C. Latham, Institute of Biological Laboratories, Department of Public Health, Boston, Mass., was available in two forms. The soluble toxoid, prepared by ammonium sulfate precipitation, was used in the Ouchterlony and LHG tests. The insoluble tetanus

toxoid suspension, partially purified by gel filtration (Latham et al., 1965) and adsorbed to aluminum phosphate, was used for injection because of its greater antigenic properties and because it was less likely to induce tolerance than the soluble form of the toxoid.

Dose

At immunization rabbits received 140 mg of tetanus toxoid (Aluminum phosphate adsorbed) per kg of body weight. This injection quantity greatly exceeded the amount necessary to elicit a response in the adult animal since the neonate appears to require a large antigenic mass in order to respond immunologically (Sterzl & Trnka, 1957 Dixon & Weigle, 1959 Riha, 1961 Bellanti et al., 1963) Control rabbits received 0.5 ml of 0 85 % physiological saline.

Route of antigen

Rabbits were administered antigen either intraperitoneally or via the palatine mucosa.

Organs

Organs excised at the appropriate ages for comparative study were the tonsils, appendix, spleen and thymus.

cal hemolysis in gel technique

A. *Coupling of antigen to erythrocytes.* Antigen was coupled to sheep erythrocytes (SRBC) according to the methods of Golub et al. (1968) and Daniels & Weigle (1968) Tetanus toxoid (0.92 mg N/ml), mixed with conjugation buffer (CB) in a 1 1 ratio, was added to the SRBC in a ratio of 3 ml of protein solution to 0 1 ml of a 5% suspension of SRBC in CB Soluble carbodiimide (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl) Ott Chemical Co., Muskegon, Mich.) (100 mg/ml CB) was used to couple covalently the given protein to SRBC (Johnson et al., 1966) and was added to the SRBC-protein mixtures in a ratio of 0.5 ml per 0.1 ml 50% SRBC in CB After incubation for 1 hour at 0–4 C, the protein-coated SRBC were washed three times in CB and diluted 1 4 in a solution of Earle s salts.

B *Cell suspensions* At specified intervals following immunization, lymphoid organs were removed and the cells were separated and washed with iced Hanks gelatin solution, buffered in a 10% $NaHCO_3$ solution to pH 7.2. These were resuspended in a 0.83% aqueous ammonium chloride solution (pH 7 0) for 5 min at room temperature to remove rabbit erythrocytes (Daniels & Weigle, 1968) Following centrifugation, the cells were then suspended at high dilutions ranging from 3×10^5 to 1×10^4 cells/0.6 ml Earle s salts to insure the occurrence of only one plaque forming cell (PFC) per plaque (Harris et al., 1966).

C. *Plaque-technique* The Jerne plaque assay (1963) was employed with modifications from the laboratories of Golub et al. (1968) and Harris et al. (1966) A suspension of lymphoid cells, 0 6 ml of an appropriate dilution, was mixed rapidly with 1 0 ml of 1.3% agarose (L'Industrie Biologique Francoise, Gennevilliers, France) in Earle s salts containing 0.1 ml of 25% suspension of antigen-coated SRBC and then was poured rapidly onto base agar plates. Following the addition of 1 ml guinea pig complement (Baltimore Biological Laboratories, Division of Bioquest, Cockeysville, Md.) diluted 1 10 in Earle's salts, each plate was incubated at 37 C for 2 hours. Two types of control plates were prepared. (*a*) those utilizing lymphoid cells from nonimmunized animals and (*b*) those using unsensitized red cells. In all tests control plates (*b*) were negative. Thus background represented plaques appearing on plates of organs from nonimmunized animals.

Samples of blood from the immunized and control rabbits were obtained by cardiac puncture immediately preceding their sacrifice. Sera were collected following centrifugation in the cold. Aliquots from each serum sample were tested by means of the Ouchterlony test and precipitation tests for precipitins to tetanus toxoid.

D *Detection of cells producing IgG anti-*

body Following incubation with complement, plaques were counted and scored as IgM producers. The method to detect IgG production was that of Dresser & Wortis (1965)

RESULTS

Pattern of immune response in tonsil

Tonsil cells from rabbits immunized at varying neonatal ages showed decreasing ability to form plaques with increasing age at both 5 and 10 day challenge (Fig. 1) However the new born tonsillar response was extremely strong and did not weaken and approach PFC values expected for a suspension of 10^4 cells in a primary reaction (Daniels & Weigle, 1968 Harris et al., 1966) until the 21st day

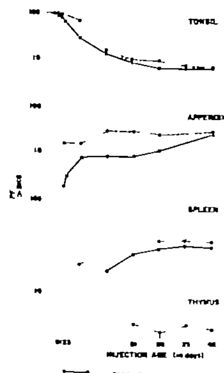


Fig. 1 Comparison of immune responses at different ages in 4 rabbit organs tested 5 days (A) and 10 days (B) after injection with tetanus toxoid via palatine mucosa. Each point is mean for 2 3 tests in which 2–4 rabbits were pooled per test. BKG back ground.

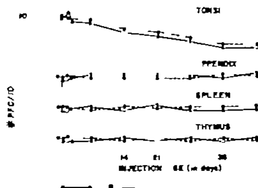


Fig. 2 Comparative background counts of 4 organs from neonatal control rabbits. Rabbits were injected via the palatine mucosa with 0.2–0.5 ml physiological saline. 5 day challenge (A). 10 day challenge (B).

Comparison of immune response at different ages in 4 rabbit organs

A. *Post 5 day test.* Until day 7 the tonsil was appreciably more responsive than the appendix, spleen and thymus (Table I, Fig. 1) By day 14 the appendix had reached the level of the tonsilar response for that age. By days 28 35 and 42, both appendix and spleen surpassed the tonsilar response Although the immune response of the spleen and appendix at their respective peaks never reached the heights of the tonsilar reaction immediately following birth, the responses were in the range expected for adult immune rabbit cells undergoing a primary response (Harris et al. 1966 Daniels & Weigle, 1968) This would seem to indicate that tolerance was not induced.

B *Post 10 day test* When 10 days were allowed to elapse after immunization, not only was the number of PFC greater for all organs (Fig. 1), but the appendix in addition to the tonsil showed immunological activity when injected hours after birth. The shape of the curves, which illustrates immunological activity as a function of age, was similar for both post 5 and post 10 day tonsil cells, indicating decreasing responsiveness. Likewise, the shape of the curve was the same for spleen cells, indicating increasing responsiveness for both challenge periods. The appendical response showed

Table I. *Comparison of immune responses of 4 organs of neonatal rabbits of varying ages (days) (LGH test)*

No. PFC/10^4 > BKG

Age at injection	Age at sacrifice	Tonsil	Appendix	Spleen	Thymus
Challenge age 5 days					
1	6	85 87 82	0, 0 0	0, 0, 0	0, 0, 0
2	7	85 90, 81	1 2, 1	0, 0, 0	0, 0, 0
3	8	70 73 68	5 3 0	0 0, 0	0, 0, 0
7	12	30, 32, 28	11 6, 8	0, 0, 0	0, 0 0
14	19	15, 15	10, 8	3, 10	0, 0
21	26	8, 10	8, 9	7 7	0, 0
28	33	7 6	12, 13	9 10	0, 0
35	40	7 8	17 18	10, 9	0, 0
42	47	7 6	30, 29	10, 9	0, 0
Challenge age, 10 days					
0	10	100, 90	8, 9	0, 0	0, 0
1	11	90 90, 91	10, 9 10	0, 0, 0	0, 0, 0
	12	92, 91 89	17 23 20	0, 0, 0	0, 0 0
7	17	67 72	21 23	4, 3	0, 0
14	24	18, 15	31, 30	7 9	0 0
21	31	10, 11	30, 30	15 16	2, 0
28	38	10, 9	4 25	13 12	1 0
35	45	8, 7	31 30	15 15	2, 1
42	52	7 6	31 33	14 13	0, 1

a Antigen-tetanus toxoid (140 mg/kg body weight)
b Antigen route–palatine mucosa.
c Each numerical value represents one test.

a climb during the first week of life a leveling for the following 2 weeks and then a subsequent climb when 5 days were allowed to elapse after immunization. If 10 days passed fore testing, the appendix showed immunocal competence at birth and then following 2-fold increase between day 1 and day 14 produced a steady level of PFC after day 14

The thymus was included in these comparative tests to represent a "lymphoid organ control" As was anticipated, the thymus revealed no significant ability to form plaques (Table I, Fig. 1) The one or two plaques observed after a 10 day waiting period might be attributed to cells that had migrated there from another site. It would thus appear that the neonatal tonsil acts qualitatively like the appendix and spleen and not like the thymus with its production of antibody· however the quantitative differences between the tonsil and these other two antibody producers immediately after birth are quite apparent.

Effect of antigen route on specific organ response

As was expected, the tonsil showed a much greater response for both 5 and 10 day intervals when antigen was injected via the palate than when it entered intraperitoneally (Figs. 3 4) Fig. 4 seems to indicate that the palatine route was not prejudicial to the spleen and even appeared to enhance the appendical response

Humoral response

When sera were tested 5 days post injection, titers were undetected until age 14 and they remained very low thereafter with animal development (Fig. 5) When 10 days were allowed to elapse following injection, sera titers were observed from rabbits injected less than a day old. The highest titers for 10 days post injection occurred from ages 14–28. It should be noted that titers were expectedly low for all neonatal ages and injection intervals when

compared with the adult or the secondary response. Bioassay of test sera was performed at the Massachusetts Public Health Biologic Laboratories. These parallel studies tested the amount of immunity to tetanus toxin conferred on mice by the test rabbit serum. Results supported the data in Fig. 5

Determination of immunoglobulin type

The plaques represented in the data are presumably the result of IgM molecules since the indirect plaque tests consistently failed to detect any significant IgG molecules. Control plates made of spleen cells from rabbits undergoing a secondary reaction did reveal additional plaques following the indirect plaque test. The lack of production of IgG in the post 10 day tests is probably attributed to the fact that all tests represented a primary response and that the animals tested were neonatal.

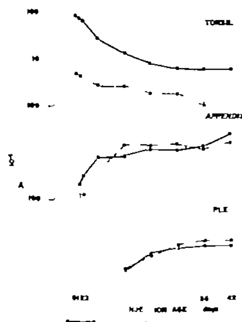


Fig 3 Effect of antigen route on specific organ response in neonatal rabbits of varying ages tested 5 days after injection. Rabbits were immunized with tetanus toxoid via the palatine mucosa (A) or via the intraperitoneal cavity (B). The thymus showed no response toward antigen *injected* via either route. Each point represents mean for 2 tests in which 1-4 rabbits were pooled per test. BKG = background.

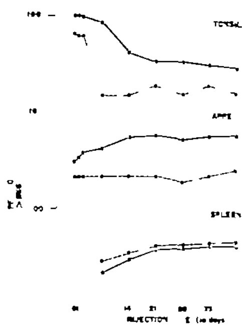


Fig 4 Effect f antigen route on specific organ response in neonatal rabbits of varying ages tested 10 days after injection. Rabbits were immunized with tetanus toxoid via the palatine mucosa (A) or via the intraperitoneal cavity (B). Each point represents mean of 2 tests in which 1-4 rabbits were pooled per test. The thymus showed the presence of 1 or 2 plaques after 21 days injection age BKG = background.

DISCUSSION

A study of the immunological behavior of the neonatal rabbit tonsil has been presented. Several factors seem to support the belief that antibody and the antibody-producing cells detected in the tonsil at the time of the test were *tonsillar in origin*. Does consistently revealed no sera precipitins to tetanus toxoid and were therefore believed to be an unlikely source for neonatal IgM antibody. Because the tonsils showed large cellular immune response upon 5 day challenge, it is thought unlikely that so many antibody-producing cells would have migrated so quickly. Thirdly antibody was not detected in the other lymphoid organs on day 1 post 5 when it was present in large abundance in the tonsil (Fig. 1). It is believed that if antibody-producing cells were migrating

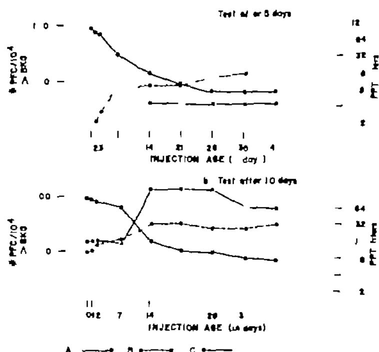


Fig 5 Pattern of cellular and humoral immune responses to tetanus toxoid. Rabbits were injected with tetanus toxoid via the palatine mucosa. Tonsil (A)- Appendix (B)- PPT titers (C) BKG = background.

from another source, antibody would have been in these organs as well as in the tonsil. Fourthly the serum titers were negative when the tonsillar response was at its peak (Fig. 5 A) hence, it does not seem likely that the antibody detected in the tonsil was coming from another source because it should have been measurable in transit in the blood. Finally the negative onse of the thymus helped to verify that dy from another source did not indiscriminately adsorb to nonimmune lymphoid cells and transform them into plaque-forming cells. Concomitant studies using bovine serum albumin in Freunds complete adjuvent as antigen yielded parallel results.

The high tonsilar response at birth is plausible since lymphocytes and macrophages have been observed in the fetal rabbit tonsil (Harrison et al. 1970). It is suggested that the decreasing immunological competence of the tonsil at days 28–42 may represent regressing tonsilar lymphoid tissue. Although the possibility of tolerance induction cannot be ignored, tolerance would have been a body phenomenon and this fact is not consistent with the data from the spleen or appendix via either injection route.

A parallel electron microscopic study has revealed that in addition to the lymphocytic cell type in the middle of plaques, epithelial cells have been found alone in plaques from tonsilar preparations up to day 14[1] and were consistent with those ages that gave the highest counts. Epithelial cells were not detected in plaques from other organs. The authors have questioned if the tonsilar epithelial cells are implicated with the background counts since the epithelial cells were detected in plaque centers during the first 2 weeks of life, concurrent with the highest background counts (Fig. 2)

It is apparent that in either the 5 or 10 day challenge tests the tonsils alone are not responsible for the humoral titers (Fig. 5) This discrepancy between tonsilar response and humoral measurements has been noted previously (Malecki, 1958 Minnik, 1965) Factors which might account for this inconsistency include: (*a*) differences between cellbound and circulat

[1] G R. Patt, E. A. Godrick & D L Patt, paper in preparation.

ing antibody (Jerne & Nordin 1963) (*b*) differences between antibody detected in the LHG reaction and an antibody detected in a humoral test tube reaction (Friedman, 1964 Hechtel et al., 1965 Minnik, 1965) or (*c*) the varying immune competencies of different lymphoid organs at different ages, and the synergistic effects of one on the others.

Under the conditions presented here, the rabbit tonsil seems to be most immunologically reactive in the early weeks of life at a time when other *future antibody-producing organs*, the appendix and spleen are either not competent or less competent. Perhaps the tonsils early peak of antibody production might be related to their vulnerable position in the organism. As the animal matures, other organs take over the tonsils early yet critical function.

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The authors are indebted to T N Harris, M.D Children's *Hospital of Philadelphia, Pa. for valuable* suggestions and to Mary Sullivan, Department of Public Health, Boston, Mass. for technical assistance.

ZUSAMMENFASSUNG

Bei Anwendung des lokalen Hämolyse-in-Gel Versuches hat sich gezeigt, dass Mandeln von Hasen auf Tetanus Toxoid ansprechen konnten wenn das Tier im Alter von 1 14 Tagen infiziert und 5–10 Tage später ausgesetzt wurde. Im Gegensatz zum Blinddarm und zur Milz, deren immunologischen Fähigkeiten zunahmen, verringerte sich bei den Mandeln während der frühen neonatalen Periode die plattenbildende Anlage. Die 10-*tägige Aussetzungsperiode* rief durchlaufend eine stärkere immune Reaktion hervor als die 5-tägige Periode und zwar in allen drei Organen im Laufe der sechswöchigen Periode. Die Palatinschleimhaut diente mit Erfolg als antigener Weg für tonsilläre Stimulierung.

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ELECTRON MICROSCOPIC STUDIES OF CAPILLARY PERMEABILITY IN NORMAL AND AMES WALTZER DEAF MICE

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Abstract At no stage after birth do Ames waltzer mice show evidence of auditory function Fluid spaces of the organ of Corti fail to develop normally Later hair cells and spiral ganglion cells degenerate and disappear These deaf mice exhibit no other morphological defects. The saccule is normal. Circulation and innervation are anatomically normal during maturation.

The tracer substance, horseradish peroxidase, escapes from capillaries by passing between endothelial cells in the peripheral circulation. Tight junctions of brain capillaries prevent penetration of this substance. In the stria vascularis, horseradish peroxidase passes out of capillaries into the fluid spaces of the epithelium, but it is stopped at marginal cell tight junctions. This is added evidence that the effective barrier between high potassium endolymph and high-sodium extracellular fluid is the marginal cell tight junction.

Histamine increases peripheral capillary permeability to horseradish peroxidase in moderate dosage. It does not facilitate brain capillary or stria vascularis penetration by this tracer

Ames waltzer mice showed no significant permeability abnormalities.

In a brief note, Green (1966) credited Schaible with the 1955 discovery of the Ames waltzer strain of deaf mice. As a personal communication to Green, Deol is quoted as saying that the inner ear of Ames waltzers resembled Shaker 2 mice.

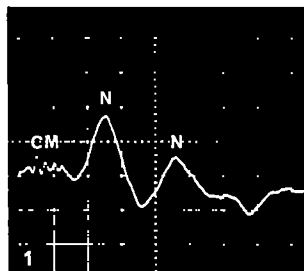
Electron microscopy has been helpful in our laboratory for study of the pathogenesis of deafness in Shaker 1 mice and Hedlund white mink. Abnormalities of innervation and probably of circulation to the organ of Corti during development were found in Shaker 1 mice (Kikuchi & Hilding, 1965 1967) Early cytological changes in the organ of Corti were found in Hedlund mink (Sugiura & Hilding, 1970 a) which resembled ototoxic antibiotic induced hair cell changes (Duvall & Wersäll, 1964 Lundquist & Wersäll, 1966, 1967)

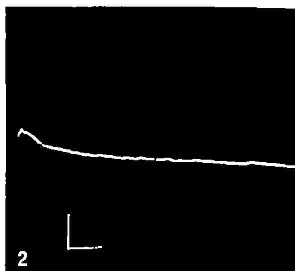
The saccule wall of Hedlund mink underwent degeneration which progressed to rupture. The stria vascularis was atrophic and its circulation abnormally sluggish (Sugiura & Hilding, 1970 b).

Because we could find no significant morphological abnormalities in Ames waltzer mice during early stages, we were led to study capillary permeability with the aid of Karnovsky's (1967) peroxidase technique. Horseradish peroxidase is an enzyme with a molecular weight of approximately 40 000 which readily escapes between capillary endothelial cells in the peripheral circulation, but will not penetrate the tight junctions of brain capillaries (Reese & Karnovsky 1967) When used as a tracer it is injected intravenously and fixed tissue blocks are incubated with 3-3-diaminobenzidine, then post fixed in osmium tetroxide An electron-dense reaction product reveals the location of peroxidase. On light microscopy a dark brown or black stain is seen.

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Fig 1 Round window electrical response to click stimulus in normal 21-day mouse showing cochlear microphonic (*CM*) and action potential (*N*) 20 µV/division, 0.5 msec division

Fig 2 Round window recording of 27-day Ames waltzer At no stage were we able to elicit either electrical or behavioral reactions in Ames waltzer mice.

Fig 3 25-day Ames waltzer organ of Corti showing absence of space of Nuel and missing outer hair cells. I this animal, the tunnel was open in the basal portion of the cochlea. Most often, the tunnel space was not open. Light microscope 590.

In this project, horseradish peroxidase was used to study histamine-induced changes in capillary permeability Other tracers have been used for studying histamine effect (Majno & Palade, 1961 Majno et al., 1967) Trypan blue was used by Nomura (1961) to demonstrate a histamine resistant barrier within the stria vascularis.

MATERIAL AND METHODS

Approximately 50 Swiss strain normal mice were used, varying in age from newborn to adult. A few fetal specimens were studied. Twenty normal mice were injected intravenously with peroxidase, of which 12 also received histamine in various doses. A colony of Ames waltzer mice was maintained. Hearing was tested by Preyer reflex and by round window recording of cochlear electrical responses (with the same equipment described by Sugiura & Hilding, 1970 a). Electrical recording was performed on 6 normal and 9 Ames waltzer mice at various stages from birth to adult. Electron microscopic observations were made on about 50 Ames waltzer mice. Peroxidase permeability studies were performed on 15 normal and 10 Ames waltzer mice of varying ages.

For routine electron microscopy the animals were sacrificed with anesthetic overdose, and the temporal bones rapidly removed. The cochleas were opened and flushed with buffered formaldehyde–glutaraldehyde and fixed for 2 hours. After washing, the specimens were osmicated for 1 hour dehydrated, treated with propylene oxide, and embedded in Epon 812. Sections for light microscopy were cut at 1.0 μ stained with toluidine blue and mounted in Permount. (Canada balsam is unsatisfactory for mounting these sections because the stain fades rapidly probably due to xylene.) For electron microscopy sections were cut with an LKB Ultratome, and stained with uranyl acetate and lead.

Horseradish peroxidase 4–7 mg was injected into the femoral vein of unanaesthetized mice. Intracardiac injection was used for mice under 5 days of age. Controls were injected with saline. At various intervals afterwards the animals were sacrificed, and the inner ears aldehyde-fixed as described above. The membranous cochleas were then dissected out, and pieces incubated in 3-3-diaminobenzidine tetrahydrochloride buffered to pH 7.6 with 0.01 H_2O_2 added, as described by Karnovsky (1967). Pieces of heart, esophagus, kidney and lung were similarly treated. After incubation for 15 min, the specimens were washed in distilled water then post-osmicated, dehydrated and embedded as described above.

To study the effect of histamine, it was given intravenously in 0.1 mg to 4 mg doses from 1 to 30 min before injection of peroxidase or applied topically to the round window in 0.2% solution. Doses larger than 1 mg were given in 1 mg increments at 5 min intervals.

RESULTS

Neither behavioral nor electrical responses could be elicited to auditory stimuli at any stage after birth in Ames waltzer mice. Our control, normal-hearing mice, gave startle responses to hand-claps 10 days after birth and recordings of round window events began to show the typical pattern by 12 days after birth. (Figs. 1 and 2 are electrical recordings from a normal 21-day mouse and a 27-day Ames waltzer.) Light and electron microscopic studies

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Fig 4 Ames waltzer embryo 16 days after fertilization showing normal cochlear duct with large swelling (*LS*) and small swelling (*SS*). Outer hair cells arise from the small swelling, and the inner hair cell from the large swelling. Spinal ganglion (*SG*) and mitotic figure (arrow). 260

Fig 5 Newborn Ames waltzer showing normal features: differentiated outer hair cells (*HC*), stria vascularis (*SV*), and intraganglionic spiral bundle (*IB*). 35

Fig 6 Ames waltzer 15 d[illegible] after birth. No tunnel of Corti. Outer (*OHC*) and [illegible]er (*IHC*) hair cell [illegible] morphologically normal. The spiral vessel (*V*) is undergoing physiological atrophy. At this stage, mice normally have [illegible] tunnel of Corti at this location (upper basal turn). 560

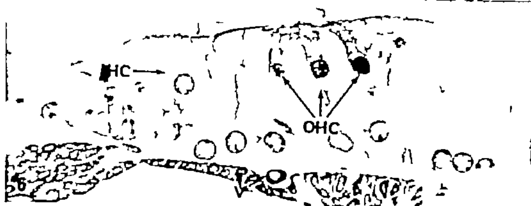
Fig 7 Adult Ames waltzer showing replacement of organ of Corti by simple cuboidal epithelium and atrophy of the spiral ganglion. The stria vascularis and the volume of endolymph seem normal. 35

Fig 8 Ames waltzer 7 day after birth, normal-appearing [illegible] pical turn. 570

Fig 9 Electron micrograph, 7-day Ames waltzer showing afferent nerve ending (*A*) at base of outer hair cell (*HC*). Some nearby nerve fibers contain vesicles, few of which are dense-core (arrows). 45


LS
SS
LSG
4


SV
IB
HC
5


IHC
OHC
6


7


8
HC
A





Fig 10 Saccule adult Ames waltzer showing its normal appearance. (Defect of sa wall and detached otolithic membrane are preparation artefacts and were seen in normal specimens.) 200.

Fig 11 Saccular sensory epithelium showing type I (*single arrows*) and type II (*double arrows*) hair cells. Basement membrane (*BM*). Toluidine blue stain, light microscopy 1 150

Fig 12 Nerve ending (*NE*) at base of type II hair cell with nucleus (*N*) indicated. Unlike most other deaf animals, Ames waltzer mice have a normal-appearing saccule by light and electron microscopy

revealed no significant difference between normal and deaf mice until about the eighth to tenth day after birth when the intercellular space and tunnel space of the organ of Corti normally develops in the basal turn Ames waltzer mice always failed to develop the space of Nuel and were late or deficient in forming the tunnel of Corti. Subsequently the organ of



Fig 13 In the stria vascularis, the tracer substance, horseradish peroxidase escaped from capillary lumen (with erythrocytes, *E*), by passing between endothelial cells and through the basement membrane (*B*) to enter fluid spaces between epithelial cells. This specimen is from 14-day Ames waltzer and shows normal permeability

Fig 14 Ames waltzer stria vascularis (*SV*) and spiral prominence (*SP*) showing normal light microscopic features Fifteen days after birth pical turn Ames waltzer 510.

Fig 15 Horseradish peroxidase permeates fluid spaces of the stria vascularis, but is limited by tight junctions (arrow) between marginal or dark cell Ames waltzer 6 days fter birth, showing normal findings 22 600.

Corti degenerated, with gradual loss of hair cell population (Figs. 3–6) In later stages, the tunnel of Corti opened in some animals (Fig. 3) No swelling or vesicular degeneration of hair cells was noted. The hair cells and nerve endings were normal by electron microscopy through 14 days (Figs 8 9) Spiral gan[illegible] cells gradually disappeared during matur[illegible]





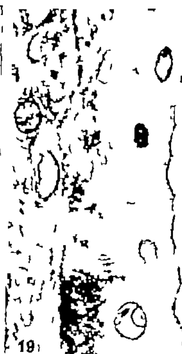


Fig 16 Normal adult stria vascularis, with horseradish peroxidase penetrating into fluid spaces of the stria epithelium. Micropinocytotic transport is not seen in the strial cells. Tracer injected 15 min before animal was sacrificed. Electron micrograph, ×7 450.

Fig 17 Light micrograph of stria vascularis of normal adult mouse injected with horseradish peroxidase 15 min before death. ×930.

Fig 18 Large histamine dosage (4 mg given in divided doses) inhibited escape of horseradish peroxidase from stria capillaries. Lumen (*L*). Electron micrograph of normal stria vascularis. ×7 450.

Fig 19 Light microscopy of normal stria showing inhibition of capillary permeability of horseradish peroxidase by 4 mg histamine. ×930.





Fig 20 Small doses of histamine increased permeability of most vessels to horseradish peroxidase. Spiral prominence epithelium was penetrated between cells up to the tight junction (sacrificed 10 min after injection of peroxidase). Topical histamine 0 2 was applied to round window Electron micrograph, 11 290.

Fig 21 Light micrograph of same specimen showing very prominent spaces in connective tissue of spiral prominence Boxes indicate approximate areas of Figs 20 and 22. 810.

Fig 22 Peroxidase penetrated connective tissue beneath the spiral prominence epithelium after escaping from vessels, after small dose of histamine. Electron micrograph, 3 950.



Fig 23 Control specimens included many tissues. This shows extracellular space (*ES*) of cardiac muscle penetrated by horseradish peroxidase 15 min after injection, from an animal treated with histamine 2 mg in divided doses. Electron micrograph, 15 470.

The sensory epithelium and membranous walls of the saccule were normal (Figs. 10–12)

The stria vascularis epithelium was nearly normal. However none of the ten specimens studied was free of possibly artefactual dark cell shrinkage. The volume of the endolymph was normal in all specimens examined.

The vascular supply to the structures of the cochlea seemed normal except for slight basement membrane thickening in stria vascularis capillaries. The spiral vessel arcade beneath the organ of Corti underwent physiological atrophy A possible abnormality of capillary permeability was suspected in the stria vascularis because of the suggestion of basement membrane thickening. This led us to use horse radish peroxidase as a tracer for permeability studies

We first repeated previous studies. When we injected horseradish peroxidase intravenously it passed between endothelial cells to enter the extracellular space in such tissues as cardiac muscle, esophageal muscle, lung, and kidney. As expected, the tracer did not penetrate past the tight junction between endothelial cells in brain. These results were consistent with the observations of Karnovsky (1967), and Reese & Karnovsky (1967).

In the stria vascularis, horseradish peroxidase penetrated between capillary cells and entered the space between the epithelial cells (Fig. 15). It was stopped by tight junctions between marginal cells and did not enter the endolymph compartment. Vessels of the spiral prominence, limbus and spiral ligament were less permeable, and little penetration was found through these capillaries except in young animals or after histamine. There was no significant difference in penetration through cochlear capillaries between normal and Ames waltzer mice.

Histamine increased the penetration of horseradish peroxidase through the vessels of the spiral prominence and ligament when given in appropriate dosage (1.0 mg I.V., or a drop of 0.2% topically on the round window). Increased permeability was found also in other systemic capillaries. No change in permeability was found at any tested dosage in brain tissue as expected.

Histamine did not increase permeability of stria vascularis capillaries at any dosage tested from 0.1 mg to 4 mg, but a reduction in penetration was noted when 2 mg or more was administered.

DISCUSSION

Failure of Ames waltzer mice to develop intercellular spaces in the organ of Corti is the only significant morphological abnormality we found that can be correlated with the deafness of these animals. Since Wada's (1923) work, it has been known that the tunnel space and other fluid spaces of the organ of Corti open at the time hearing develops. Alford & Ruben (1963) carefully correlated anatomical and physiological development in mice and found that electrical and behavioral responses could be elicited about 10 days after birth when the organ of Corti began to achieve maturity. Their findings were confirmed by Kikuchi & Hilding (1965) who also found that efferent innervation occurred at this critical time.

Unlike most other deaf animals, Ames waltzer mice have normal saccular sensory epithelium. Schuknecht et al. (1965) recently discussed many of the different forms of deafness that exhibit cochleo-saccular degeneration. We have been unable to find another example of an hereditarily deaf animal strain with documentation of normal saccular epithelium. Endolymph volume and the stria vascularis appeared normal. Capillary permeability as tested with the tracer horseradish peroxidase, was normal.

According to Simon et al.'s (1970) concept of endolymph formation, the apical junction between dark cells should be impermeable, but the space between cell processes should be in limited communication with circulating blood plasma. The fact that horseradish peroxidase does pass from strial vessels into the intercellular spaces of the stria epithelium and that it is stopped at the junctions near endolymph fits Simon et al.'s theory.

Histamine in appropriate dosage increased the permeability of systemic capillaries to horseradish peroxidase. This was true of the vessels of the spiral prominence and spiral limbus as well as esophagus, heart, etc. As previously reported by Nomura (1961) who used trypan blue as a tracer, permeability of stria vessels was inhibited. No change of permeability was found in cerebral capillaries.

ZUSAMMENFASSUNG

Das Gehörorgan von Ames Waltzer Mäusen scheint zu keiner Zeit nach der Geburt zu funktionieren. Die Räume für Flüssigkeiten in dem Corti Organ wachsen nicht normal auf. Die Haarzellen und Spiral Ganglienzellen erschienen und verschwinden später. Diese tauben Mäuse haben keine anderen morpholog-

Acta

Fehler Das Saccule ist normal. Zirkulation und Innervation scheinen während des Wachsens normal.

In der peripheren Zirkulation, verschwindet der Spurenstoffanzeiger Meerrettich Peroxydases, von den Kapillaren und sickert zwischen den Endothelzellen durch. *Die enge Zusammenfügung der Gehirnkapillaren verhindert das Durchdringen dieses Spurenstoff anzeigers.* In der Stria Vascularis verlässt Meerrettich Peroxydases die Kapillaren und fliesst in die Flüssigkeitsstellen des Epithels; aber es kann die enge Zusammenfugung der Randzellen nicht durchdringen. Weiterhin steht fest, dass die enge Zusammenfügung der Randzellen die wirkende Barriere zwischen dem hohen-kalium-Endolymph und der hohennatrium extrazellularen Flüssigkeit ist.

Die Durchdringbarkeit der peripheren Kapillaren durch Meerrettich Peroxydases wird durch Histamine erleichtert. Dies ist nicht der Fall bei Gehirnkapillaren oder der Stria Vascularis.

Bezüglich der Durchdringbarkeit sind bei Ames Waltzer Mäusen keine besonderen Abnormitäten vorgefunden worden.

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LESIONS LOCALIZED TO THE EIGHTH NERVE AND ABNORMAL THRESHOLD ADAPTATION

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Abstract. A group of 5 patients was studied in each of whom lesion was produced during neurosurgical procedure in which damage was limited to the fibers of the eighth nerve. Preoperative and postoperative studies of ATA would indicate that in those patients the ATA was specifically related to the conductive function of the eighth nerve fibers. Other studies have shown that ATA can be produced by lesions of higher order neurons (see Parker et al 1962, 1968). Extent of ATA in these lesions can be independent of threshold sensitivity and discrimination score.

The purpose of this paper is to describe the effects of a lesion confined to the fibers of the eighth nerve and the occurrence of abnormal threshold adaptation (ATA). By the term abnormal threshold adaptation is meant the complete fade-out of a tone presented to the test ear under the exact conditions defined by Carhart in his description of the tone decay test (1957) All cases in this series exhibited ATA as opposed to tone perversion. The former is to be differentiated from poststimulatory threshold shifts and suprathreshold per or poststimulatory threshold shifts.

Interest in this phenomenon occurred as early as 1893 when Gradenigo published a paper on the Clinical Signs of the Affections of the Auditory Nerve" Attention was refocused on it by Hood in 1950 and has subsequently been investigated and discussed by many other authors. In most instances, the extent of damage to the auditory system in the cases studied could not be well defined. For example, in cases of eighth nerve tumors showing extensive ATA, effects on the vascular supply to the inner ear caused by compression of the internal auditory artery or effects on the nuclear area caused by pressure on the brain stem, could not be excluded as partial causes of the abnormal auditory effects. This has also been true in regard to other cerebellopontine angle tumors, apparent viral neural infections, and other pathologic conditions

We have had the opportunity to study a group of patients in each of whom a well-localized lesion confined to the eighth nerve has been produced. These are all cases of organic involuntary uncontrollable hemifacial spasm for which the neurosurgeon had performed a neurolysis of the seventh nerve intracranially In this procedure, a suboccipital craniotomy is performed, and the cerebellum elevated to expose the cerebellopontine angle. The eighth nerve is then isolated from the seventh and is gently retracted in order to inspect the seventh nerve along its entire length (Fig. 1) A thorough review of the operative notes and discussion of these cases with the operating neurosurgeon indicated that no damage had been done to the internal auditory artery or to the lateral pons, thereby reasonably eliminating the possibility of damage to the vascular supply of the inner ear or to the nuclear area of the

Fellow Department of Otolaryngology Cleveland Clinic.

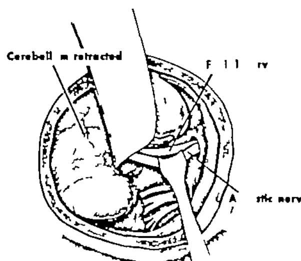


Fig 1 Suboccipital appr h he cerebellopontine angle and xposure of the t seventh nerve showing the retraction of h r ranial nerve fibers which is respons) no mal post operative auditory findin

auditory tract. This 1 n almost ideal experimental situat p ducing an isolated lesion of the eighth n b s a coinciden-tal side effect to the 1 p pose of the sur gical procedure Fi are pr sented in each of whom pre nd stoperative studies were done

PROCEDURES

Each patient received a complete otologic ex amination preoperatively after which audio-metric tests were performed. A conventional pure tone threshold was first determined, then a tone decay test was done strictly adhering to the Carhart technic (1957) Speech reception thresholds were determined at the level where the subject heard 50% of five-voice spondee words correctly Speech discrimination scores were obtained by presenting a list of Harvard phonetically balanced words at an intensity of 30 dB above the speech reception threshold.

RESULTS

None of the 5 patients in this study had more than mild abnormalities of threshold sensitivity preoperatively Three patients had significant ATA preoperatively at 8 000 Hz only In 2 patients, it was found on the side of the hemi-facial spasm. In the other patient, ATA was found in both ears at 8 000 Hz. Discrimin-

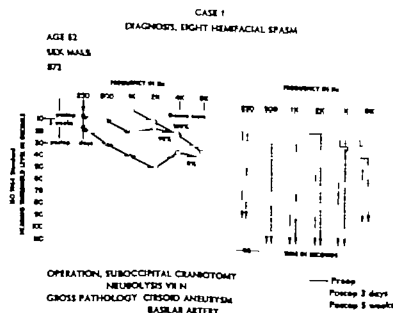


Fig 2 Pre- and postoperative studies in case 1 Note appearance and retention of complete ATA, moderate depression and partial recovery of PTT sensitivity and reduction of the discrimination score to 0 percent followed by good recovery despite persistence of complete ATA.

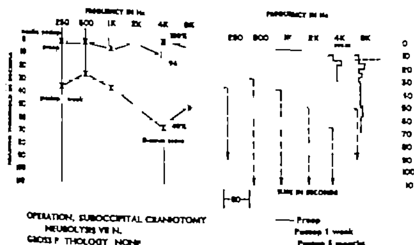


Fig. 3. Pre- and postoperative studies in case 2. Note appearance of complete ATA followed by complete disappearance, and moderate depression of PTT sensitivity and discrimination score followed by complete recovery of both.

ion scores were 96% or better preoperatively n all patients.

Postoperatively in 4 of the 5 patients (Figs. 3–6) pure tone threshold changes would be classified as mild to moderate, and in the other moderately severe. The Carhart test demonstrated complete ATA at all frequencies on the affected side in 4 patients, and moderate but significant ATA at all frequencies in the other patient. In the latter patient, the discrimination score was reduced from 100% to 76%. In 3 of the others discrimination score was reduced to 0 postoperatively and in the fifth patient to 40%.

A second postoperative follow-up study was obtained of 2 of the 5 patients. In case 1

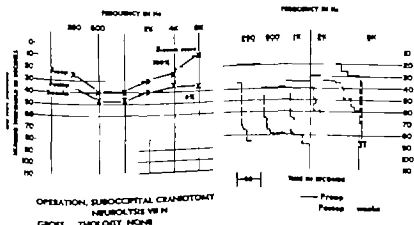


Fig. 4. Pre- and postoperative studies in case 3. Note appearance of incomplete ATA below 8 kHz, mild depression of PTT sensitivity and mild decrease of discrimination score.

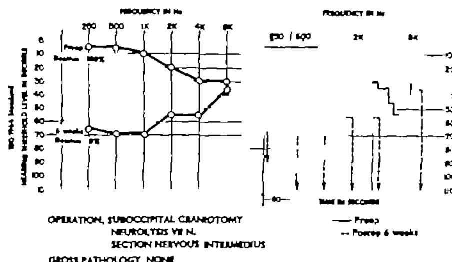


Fig 5 Pre and postop tive studies in case 4. N appearance of complet ATA, moderately sever depression of PTT se tivity for low tones, change in discriminatio score from 100 to 0 p cent.

(Fig. 2) the pure tone threshold improved almost to normal 5 weeks postoperatively even though ATA was complete at all frequencies. Despite the latter the discrimination score improved to 92% In case 2 (Fig. 3) reexamination 5 months postoperatively showed complete recovery of the pure tone threshold, co plete absence of ATA, and a discriminati score of 100%

In 2 of the 5 patients, a cirsoid aneurys of the basilar artery was discovered. In t other 3 patients, no pathologic lesion w

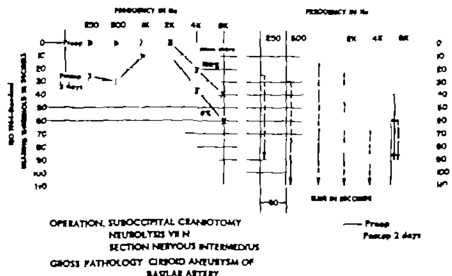


Fig 6. Pre- and postoper tive studies in case 5 Not appearance of complete ATA below 8 kHz, rel tively mild PTT sensitiv depression, but complet loss of discrimination abil ity

fond. In 2 patients, the nervus intermedius was sectioned. In the other 3 only lysis or freeing of the seventh nerve alone was performed. Neither section of the nervus intermedius nor the presence of the cirsoid aneurysm altered the pattern of the postoperative test results. There were no auditory changes on the nonoperated side.

COMMENT

It appears reasonably certain that we are dealing with the effects of a specific and localized lesion of the eighth nerve fibers caused by resection of the nerve a matter of a few millimeters, and that the adverse auditory effects are specifically related to this lesion and to disorders of nerve conduction. In case 2 this alteration of nerve function was obviously only physiologic and temporary as complete recovery of all functions occurred. In case 1 recovery of pure tone threshold and discrimination ability occurred in the presence of continuing complete ATA, demonstrating partial recovery. We have other case data in our files not presented here in detail because of lack of preoperative studies, in which little or no recovery of ATA occurred as established by postoperative examinations over a 4 year period.

ZUSAMMENFASSUNG

Eine Gruppe von 5 Patienten wurde untersucht bei denen der VIII. Hirnnerv bei einem neurochirurgischen Eingriff beschädigt wurde. Die Prüfung der abnormen Schwellenadaptation vor und nach der Operation ergab, dass bei diesen Patienten die abnorme Schwellenadaptation in spezifischer Beziehung zur Leitfähigkeit der Fasern des VIII. Hirnnervens stand. Andere Studien haben gezeigt, dass abnorme Schwellenadaptation durch Schädigung höherer Neurone verursacht werden kann (Parker et al. 196_, 1968). Das Ausmass der abnormen Schwellenadaptation kann bei diesen Schädigungen unabhängig von der Schwellenempfindlichkeit und der Unterscheidungsschwelle sein.

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PATTERNS OF PURETONE LOSS IN PRESBYCUSIS

A Sequential Study

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Abstract. A retrospective study of the changes occurring in sequential puretone audiograms of patients with presbycusis was carried out. The average period under review was 9 4 years in the 25 patients selected. 92% of the ears under study retained the pattern of audiogram initially noted. The possible explanations of the changed pattern in the other cases is discussed.

Various types of hearing loss have been ascribed to discrete pathologic changes at different sites of the inner ear (Schuknecht, 1964) No known study however purports to prove the constancy of puretone patterns in presbycusis over prolonged periods of time. This thought occurred to us during discussions of our temporal bone histopathologic study of presbycusis, and the present study was set up to determine the consistency of these patterns over a period of time.

SUBJECTS

All available coded audiologic records at Sunnybrook Hospital, Toronto, were searched for patients with bilaterally symmetrical sensorineural hearing loss associated with ageing who had had at least two audiograms. Subjects with a definite history of stimulation deafness or sensorineural hearing loss secondary to known etiologic factors, e.g. drugs, were excluded, as were those with mixed or purely conductive hearing losses. The minimum follow-up period considered was one year (see Tables I and II)

The records of 25 suitable patients were obtained and studied (i.e 50 ears) 23 patients were males and only two females. (This was formerly a Veteran s Hospital serving a predominantly male population group.) The range of ages was 52 to 89 with an average of 73.

EQUIPMENT

All tests were performed in sound-proof booths with reliable calibrated clinical audiometers using conventional techniques for pure tone testing. The early tests in each case were usually ASA and the more recent ISO calibrated but no correction was made for this factor as it was the pattern of the result that we were interested in here

FINDINGS AND DISCUSSION

The audiograms were classified by a modified (Dayal et al. 1970) Carhart s (Carhart, 1945) method. Briefly descending curves had greater than 10 dB losses in the higher frequencies, while flat curves had less than 10 dB difference between different frequencies (esp. 500, 1 000, 2 000 cps.) The multiple audiograms for each

Table I

	Range	Average
Period under review	1 y 2 mo.–24 y	9 y 5 mo.
No. of audiograms per patient	2–7	3

Table II

No. of patients followed (years)	over 2–20 over 5–14 over 10–10 over 20–5

Table III

Frequency (Hertz)	Loss in dB Minimum	Maximum	Average
500	5	30	10
1 000	5	30	10
2 000	5	45	10

patient were charted on the same form and studied to yield the information reported below Fig. I shows the hearing tests on one ear of a patient over a 19-year period. The descending pattern is unchanged The average period of study was 9 years 5 months and there was an average of *three audiograms* per patient. However a substantial number of our patients (10) were followed for 10 years or more (Tables I and II) Table III shows the minimal, maximal and average changes in hearing at 500, 1 000 and 2 000 hertz.

The analysis of audiologic patterns revealed that 88% of the patients had descending and

Table IV

	Puretone patterns Descending	Flat
total	88	12
stable	82	10
% changing	6 (These became Flat")	2 (These became descending)

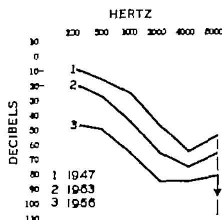


Fig 1 Showing pattern of hearing loss p ... over 19 years.

12% flat type of curves at the initial hearing test. There is an apparent discrepancy in the % of descending and flat patterns in the present study as compared to the previous study (65% descending and 31% flat, Dayal et al 1970) This is because of the selective criteria for patient selection in this paper—only patients with multiple audiograms were considered whereas in previous publication consecutive records were taken for analysis.

Of the descending curves 82% remained unchanged while 6% became flat over the period of investigation. While of the flat curves, 10% remained stable and only 2% became descending (Table IV) Thus, it is seen that the vast majority of ears had a constant pure tone pattern of hearing loss, but, a small number do change The implication of this finding may be twofold.

(*a*) In the majority of ears the consistency of the pattern of puretone hearing loss would suggest that the site of pathology causing it has remained unchanged over the years.

(*b*) In a small percentage (8%) the change of the pattern of puretone loss might indicate a change in site of primary pathology—or in other words, two sites of pathology may account for the changed pattern and that one has been added into the other over a period of years. These ears, therefore, may have sites of pathological changes to

the final hearing loss. A search of the records of patients whose audiological pattern had changed, failed to reveal any relevant factors in producing such change.

Hearing change in dB over the period of the study is shown in Table III. Of interest, is the fact that there was no consistency in the magnitude or rapidity of hearing loss in flat or descending curves, with time in these patients

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ZUSAMMENFASSUNG

An Patienten mit Presbyakusis wurde eine rückblickende Studie über die Veränderungen in aufeinander folgenden Audiogrammen mit reinen Tönen durchgeführt. Die durchschnittliche Beobachtungszeit an den [illegible]5 gewählten Patienten betrug 9.4 Jahre. 97% der geprüften Ohren behielten das bei der Erstuntersuchung gefundene Audiogramm-Muster. Bei Fällen, bei denen es zu einer Musteränderung kam, könnte zu der ursprünglichen Schädigung eine zweite hinzugekommen sein.

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HUMAN TEMPORAL BONE FINDINGS POST STAPEDECTOMY

A Review of Ten Cases

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Abstract. Ten temporal bones were obtained at varying intervals following stapedectomy They have been studied regarding middle and inner ear changes f ter this type of surgery The review of these ten temporal bones confirms the clinical impression that the present surgical techniques used in stapedectomy routinely produce little middle and inner ear reaction

During the past decade, stapedectomy has been extensively used in the surgical treatment of clinical otosclerosis. Various surveys (Hough, 1966 Kerth, 1967) comprising a large number of cases, have shown that stapedectomy can consistently achieve closure of the air–bone gap with permanent results The risk of inner ear damage, evidenced by a deterioration of bone conduction although relatively small (Kaufman & Schuknecht, 1967) is undeniably a possible consequence of this operative procedure.

The purpose of the present study is to increase our knowledge of some of the long-term middle and inner ear changes that might result from stapedectomy

MATERIAL

This study consists of the review employing microscopy of 10 temporal bones from patients who had undergone stapes surgery and subsequently died at varying intervals following this surgery These specimens are from the temporal bone collection at the Massachusetts Eye and Ear Infirmary Some of the findings in specimens have been reported previously (Rutledge et al., 1963 Schuknecht et al., 1964 Wolff et al. 1968) and 5 of them are hitherto unreported.

The specimens are from 2 females and 8 males. The age of the patients at the time of death varies from 41 to 81 years. The interval following stapes surgery ranges from 9 months to 5 years, the average being 22.5 months. Table I shows the type of prosthetic device following stapedectomy

The stapedectomies were performed by 9 different surgeons using 5 different techniques thus permitting a wide range of histological observations and correlations

FINDINGS

Table II illustrates the pathological findings in 10 temporal bone specimens. The areas of histologic change can be classified and discussed as follows.

Incus

The incus appeared to tolerate the wire prosthesis with little reaction. In 8 cases ranging from 36 to 60 months after surgery there were no visible changes in the bony framework of the incus In 2 cases, 21 and ..5 mx

Table I *Stapedectomy prosthesis*

Type of prosthesis	No. of cases
Wire, fatty tissue	6
Stainless steel piston	2
Wire, gelfoam	1
Polyethylene strut	1
Total	10

Table II *Temporal bone findings attributable to stapedectomy*

Pathology	No. of cases
Partial bony resorption of incus	2
Slight middle ear adhesions	3
Inadequate seal of the oval window	1
New bone formation in the oval window	3
Bone fragments in the vestibule	3
Organ of Corti hair cell loss	0
Spiral ganglion loss	0
Abnormalities of the macula	0
Abnormalities of the cupula	0

post stapedectomy there was some slight bone resorption at the side of the contact with the prosthesis (Fig. 1) In attempting to evaluate the significance of these changes, 31 normal temporal bones were examined and 3 were found to have areas of resorption of the same agnitude in the long process of incus. Similar rvations were previously reported by Bel cel & Wolff (1966)

This study indicates that one must be cautious in attributing all the bone changes in the long process of the incus solely to the wire prosthesis When bone reaction does occur in the incus, it is mild and does not appear to affect the function of the ossicles as a part of the sound transmission apparatus. As suggested by Schuknecht, it appears that a firmly attached wire prosthesis is well tolerated by the incus and any osteitus which might occur is more likely to be caused by a loosely crimped wire.

Middle ear adhesion

There was no major inflammatory reaction in the middle ear as a result of stapes surgery In one specimen, there was a fibrous band between the tympanic membrane and the incus. This adhesion was not in contact with the wire prosthesis and is most likely a result of tympanotomy and not the prosthesis.

In 2 cases there were some fibrous adhesions surrounding the wire in the region of the oval window niche (Fig. 2) In both these cases the footplate was markedly thickened by otosclerosis and in order to perform the stapedectomy a drill had to be used. The adhesions appear to be the result of surgical trauma and not related to the type of prosthesis used.

The minimal middle ear changes seen in our 10 human temporal bones contrast with the marked adhesions found following middle ear



Fig 1 A. H 56 years. 1 months after stapedectomy with fat-wire prosthesis. Resorption of the bone in the long process of the incus near the attachment of the wire.



Fig 2 B A., 65 years, 9 months post-stapedectomy with fat-wire prosthesis and drilling out of the otosclerotic bone in the oval window The fatgraft is replaced by dense fibrous tissue surrounding the wire. A fibrous band can be seen between the fibrous tissue around the wire and the mid wall. There is some new bone format window region. The arrow indicates the wire.

surgery in experimental animals (Paparella, 1967). The reason for this must be species-specific.

Seal of the oval window

In only one of 10 cases was there inadequate closure of the oval window by the tissue graft and this occurred following use of a polyethylene strut. This case has been reported in detail by Rutledge et al. (1963) and similar cases have also been reported by Matz et al. (1968) and Wolff (1964) In these 3 reported cases, meningitis occurred following use of the polyethylene strut and this type prosthesis has subsequently been abandoned by most surgeons.

Excluding use of the polyethylene strut, it appears that the connective tissue prosthesis provides good closure of the oval window. In 9 of our cases, the oval window was closed by an adequate connective tissue membrane however the fibrous membrane was somewhat thinner after gelfoam use than after fat substitution. In most cases the fat graft was completely replaced by fibrous tissue but occasionally one could find isolated lipoid cells (Fig. 3) The presence of fatty tissue did not appear to have any effect on the hearing result following stapedectomy

New bone formation in the oval window

In 3 cases there was microscopic evidence of new bone formation in the oval window nich. In 2 of these cases, the area of new small however in one case new bone

Fig 3

Fig 4



Fig 5 C. K., 63 years, 18 months post-stapedectomy with fat-wire prosthesis. Bony dust can be seen on the saccular and utricular wall.

was abundant. In this case a drill-out stapedectomy was performed and a fat–wire insert used. The hearing improved but there was incomplete closure of the air–bone gap. Microscopic examination (Fig. 4) reveals a deep oval window niche with a remnant of footplate and new bone growth around particles of bone dust. New bone formation has also been reported following stapedectomy in experimental animals as well as in human temporal bones (Wolff 1964 Singleton & Schuknecht, 1959 Lindsay 1961)

Vestibule

In 3 cases there was evidence of partial displacement of the bony footplate into the vestibule. In one of these cases particles of bone were found resting on the walls of the saccule and utricle (Fig. 5) however in all these cases there was no evidence of reaction to the bone in the vestibule The vestibule and its sensory structures appear very resistant to the surgical trauma of stapedectomy and seem to tolerate bone fragments without reaction.

Fig 3 A. H 58 years, 18 months post-stapedectomy with fat–wire prosthesis. The fat graft is almost completely replaced by loose fibrous tissue. However some lipoid cells remain The arrow indicates the position of the wire

Fig 4 G R., 68 years, 48 months post-stapedectomy with fat–wire prosthesis. The graft is rep.. fibrous tissue Before the removal of the drill-out was performed. There is new tion, especially around bony chips.

Vestibular sensory structures

In all 10 temporal bones, no histologic abnormality of the vestibular sensory organs could be detected. In all cases, the maculae of the saccule and utricle as well as the cupulae of the semicircular canals were histologically normal. In one case the patient complained of marked vertigo following stapedectomy however examining the temporal bone obtained 10 months postoperatively failed to reveal any abnormality of the vestibular sensory organs. The etiology of the vertigo remains unexplained

Cochlear labyrinth

There was no evidence of hair cell loss in any of the 10 cases which could be attributed to the operative procedure In one case there was extensive hair cell loss and ganglion loss throughout the cochlea of the operated ear however the same changes were found on histologic examination of the non-operated ear It should be noted that there was no change in the preoperative and postoperative bone conduction audiograms in any of these patients. This contrasts with the usually reported 1–2% incidence of sensorineural loss post stapedectomy (Kaufman & Schuknecht, 1967)

The histologic findings in our very limited also contrast with those seen in post stapedectomized experimental animals (Hohmann 1962 Paparella et al. 1966) We found no evidence of serous or fibrinous labyrinthitis Endolymphatic hydrops and hypotonic atrophy of the organ of Corti likewise was not present.

In at least one of our cases, an acceptible quantity of blood was noted to have entered the vestibule at the time of surgery Microscopic examination of the temporal bone obtained 21 months post-stapedectomy failed to disclose any trace of blood and no abnormalities of the sensory structures could be detected. This agrees with the findings of Schuknecht et al. (1964 1965) that blood in itself causes no injury to the auditory and vestibular sensory organs.

There was no evidence of spiral ganglion loss except in the ear mentioned above in which similar findings were seen in the non-operated ear

ZUSAMMENFASSUNG

Zehn Schläfenbeine wurde in verschiedenen Zeitabschnitten nach einer Stapedektomie histologisch untersucht. Die Veränderungen im Mittelohr und Innenohr wurden analysiert. Diese Analyse hat die klinische Eindrücke bestätigt in dem Sinne dass nach der *routinemässigen Stapesoperationen nur unbedeu*tende Reaktion in Mittel- oder Innenohr zu finden ist.

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DÜNNSCHICHT-CHROMATOGRAPHISCHER NACHWEIS VON GEWEBELIPIDEN AUS DER MEERSCHWEINCHENSCHNECKE

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(Eingegangen am Juli 7 1970)

Abstrakt Die Lipide aus der Meerschweinchencochlea wurden unter Anwendung einer neuen Nachweismethode (Schindler & Wolf, 1968) dünnschicht-chromatographisch untersucht. In den Geweben des Ductus cochlearis einschließlich des ganzen Ligamentum spirale konnten folgende Neutralfette als Hauptfraktionen nachgewiesen werden: Triglyceride, freie Fettsäuren und Cholesterin. Daneben sind in geringer Menge Cholesterinester und wahrscheinlich Kohlenwasserstoff enthalten. In den gleichen Geweben konnten folgende Phospholipide nachgewiesen werden: Kephaline Lecithine und Sphingomyeline. Eine weitere Fraktion im Chromatogramm konnte bisher nicht identifiziert werden. Die Gewebe des Modiolus enthalten darüber hinaus als Hauptfraktion noch Cerebroside und eine weitere bisher nicht identifizierte Fraktion. Die getrennte Untersuchung der einzelnen Schneckenwindungen ergab daß alle Lipidfraktionen, die im gesamten Ductus cochlearis enthalten sind, auch in den einzelnen Windungen vorliegen. Die Menge der Neutralfette nimmt in der Schnecke von der Basalwindung zur Spitzenwindung hin zu. Dieser Befund entspricht dem Ergebnis histochemischer Untersuchungen. Die mögliche funktionelle Bedeutung dieses Befundes wird diskutiert.

Das Vorkommen von Lipiden und Lipasen in cochlearen Strukturen und in der Perilymphe macht es wahrscheinlich, daß die Lipide neben ihrer strukturellen und funktionellen Bedeutung in der Schnecke auch direkt zur Energieversorgung des Innenohres beitragen. Die Klärung der Frage, in welcher Weise und in welchem Maß Lipide für die Innenohrfunktion Bedeutung haben, bedarf umfassender funktioneller Untersuchungen, die bisher wohl wegen besonderer methodischer Schwierigkeiten noch nicht vorliegen

Die Voraussetzung für funktionelle Untersuchungen ist die Kenntnis des normalen Lipidspektrums in cochlearen Geweben und Flüssigkeiten. Bisher wurde die Lipidverteilung in Innenohrgeweben fast ausschließlich mit histochemischer Methodik untersucht (Chou, 1961 Rauch, 1964 Schätzle & Westernhagen, 1966, 1967 Gerhardt & Pieplow 1967 Schätzle et al., 1967 u. a.) Eine weitgehende Differenzierung der Gewebelipide in die einzelnen chemischen Verbindungsklassen gelingt jedoch histochemisch nicht.

In jüngster Zeit haben erste Untersuchungen gezeigt, daß auch die Dünnschicht-Chromatographie für die Untersuchung von Lipiden sowohl in Innenohrgeweben (Schiff & Christiensen-Lou, 1967 Gerhardt et al., 1968 Esser 1969) als auch in Innenohrflüssigkeiten (Schindler & Wolf 1968 Wießmann, 1969 Wolf 1969) anwendbar ist. Eine Aussage über die Lokalisation dünnschicht-chromatographisch getrennter Gewebelipide in bestimmten cochlearen Strukturen ist möglich, wenn die entsprechenden Gewebebereiche isoliert und ihre Extrakte chromatographiert werden. Die dazu erforderliche Probemenge ist abhängig vom Lipidgehalt der betreffenden cochlearen Struktur und von der Empfindlichkeit der Nachweismethode

Der bisher übliche Nachweis für die dünnschicht-chromatographisch getrennten Lipide mit spezifischen Sprühreagenzien aber auch durch Verkohlen mit Schwefelsäure oder Chromschwefelsäure erfordert noch relativ

große Probemengen. Durch Anwendung der kürzlich von Schindler & Wolf (1968) entwickelten neuen empfindlicheren Nachweismethode kann die erforderliche Probemenge wesentlich reduziert werden. Wir haben deshalb unter Anwendung dieser Methode unsere früheren dünnschicht-chromatographischen Untersuchungen von cochlearen Gewebelipiden fortgesetzt. Über die Ergebnisse wird nachfolgend berichtet.

MATERIAL UND METHODE

Gewinnung der Lipide

200 bis 300 g schwere Meerschweinchen wurden in Äthernarkose dekapitiert, die Felsenbeine entnommen und die Bulla breit eröffnet. Unter dem Auflichtmikroskop wurde die Schneckenkapsel vorsichtig abgetragen. In einer ersten Versuchsreihe untersuchten wir getrennt a) die Gewebe des Ductus cochlearis (Ligamentum spirale, Cortisches Organ und Limbus spiralis) und b) die im Modiolus enthaltenen Gewebe einschließlich des Anfangsteils des Hörnerven.

Dazu wurden zuerst die Gewebe des Ductus cochlearis herauspräpariert und dann der Modiolus herausgebrochen.

In einer zweiten Versuchsreihe untersuchten wir getrennt die Gewebe des Ductus cochlearis der einzelnen Windungen (1. bis 4. Windung). Dazu wurden die Gewebe der einzelnen Windungen gesondert präpariert.

Zur Extraktion wurde das isolierte Material der einzelnen Präparationen von mehreren Schnecken in ungefähr je 3 bis 5 ml Chloroform-Methanol 2 : 1 (Folch et al., 1957) gelegt und über Nacht unter Stickstoff stehen gelassen. Das Lösungsmittel wurde dann im Vakuum unter leichtem Erwärmen im Wasserbad abdestilliert und der Rückstand in 20 μl Chloroform-Methanol 2 : 1 pro extrahiertes Material einer Schnecke gelöst. Eine Reinigung der Lipidextrakte nach Folch et al. (1957) erwies sich als nicht notwendig (Esser 1969).

Dünnschicht-Chromatographie

Als Fließmittel wurde für die Trennung der Neutralfette Petroläther (Kp. 60–70 C)-Diäthyläther-Eisessig 85 : 15 : 2 verwendet, da bei dem ursprünglich angewandten Volumenverhältnis 90 : 10 : 1 (Gerhardt et al. 1968, Esser 1969) die R_F-Werte der Fettsäuren und des Cholesterins zu niedrig lagen. Für die Trennung der Phospho- und Glykolipide wurde Chloroform-Methanol-Wasser unverändert im Volumenverhältnis 65 : 25 : 4 verwendet.

Der Nachweis der dünnschicht-chromatographisch getrennten Lipide wurde nach der von Schindler & Wolf (1968) angegebenen Methode durchgeführt. Dabei wird Kieselgel-G unter Zusatz von 5 % Kaliumtetrathionat auf 9 × 12 cm große feuerfeste Rasotherm-Glasplatten (VEB Jenaer Glaswerk Schott & Gen.) in der üblichen Weise aufgetragen und aktiviert. Für eine Platte wurden 2 g Kieselgel-G + 5 ml 2 %ige frischbereitete Kaliumtetrathionat-Lösung verwendet. Zur Sichtbarmachung der chromatographierten Lipide wurden die entwickelten Chromatogramme nach dem Abdampfen des Fließmittels mit einer sauberen feuerfesten Glasplatte abgedeckt und bis zur Schwärzung der Substanzflecken auf einer elektrischen Kochplatte erhitzt.

Die Identifizierung der einzelnen Lipidfraktionen war in den vorangegangenen Untersuchungen (Gerhardt et al. 1968, Esser 1969) mit spezifischen Sprühreagenzien und Vergleichsubstanzen in der üblichen Weise durchgeführt worden. Zur Kontrolle wurden jetzt Vergleichssubstanzen nach der hier beschriebenen Methode noch einmal mit chromatographiert.

Um reproduzierbare Chromatogramme zu erhalten, erwies es sich als notwendig, die Chromatographie in einem sog. „Sandwich-Kammersystem" (Jänchen, 1964) durchzuführen, welches vor dem Entwickeln der Chromatogramme zusätzlich mit dem Fließmitteldampf gesättigt wurde. Die verwendete Sandwich-Kammer bestand aus der eigentlichen D schichtplatte mit den aufgetragenen Su und einer zweiten Dünnschichtplatte

platte. Von beiden Dünnschichtplatten wurde an den Seiten und an der Oberkante ein ca. 1 cm breiter Streifen des Sorptionsmittels entfernt. Als Dichtungs- und Distanzstreifen diente ein entsprechend zurechtgeschnittener Papprahmen (etwa 5 mm breit und 3 mm stark). Vor dem Chromatographieren wurde die Sorptionsschicht der Deckplatte mit dem Fließmittel getränkt. Danach wurde die Sandwich-Kammer zusammengesetzt und, durch Federklammern zusammengehalten, in eine übliche Entwicklungskammer gestellt, wo die beiden Dünnschichtplatten mit ihrer Unterkante in das Fließmittel eintauchten.

Zur Verbesserung des Trenneffektes wurden die Chromatogramme mit sog. „Keilstreifen" (Stahl, 1967) versehen (s. Abb. 1 bis 4).

ERGEBNISSE

Dünnschicht-chromatographische Trennung

Durch die Verwendung der Sandwich-Kammer und der Keilstreifen-Technik wurde der Trenneffekt und die Reproduzierbarkeit der Chromatogramme wesentlich verbessert. Bei unseren früheren Untersuchungen war es durch die unterschiedliche Flüchtigkeit der verwendeten Fließmittelkomponenten zu Entmischungserscheinungen gekommen, die zur Bildung einer zweiten Fließmittelfront führten und die Trennung erheblich beeinträchtigten. Solche Störungen treten auch auf, wenn die Sandwich-Kammer nicht dicht schließt. Die Chromatogramme laufen dann häufig schräg, und es kann ebenfalls zur Ausbildung einer zweiten Fließmittelfront kommen. In dieser Front liegen bei der Trennung der Neutralfette die Fettsäuren und bei der Trennung der Phospho- und Glykolipide die unterhalb des Cholesterins lokalisierte bisher nicht identifizierte Fraktion mit etwas erhöhtem R_F-Wert (s. Abb. 2 *c*). Außerdem tritt bei der Trennung der Phospholipide häufig ein unterhalb des Sphingomyelins liegender Fleck auf (Abb. 2 *c*), dessen auf Grund des R_F-Wertes mögliche Identität mit Lysolecithin jedoch fraglich ist.

Wir vermuten, daß es sich bei diesem Fleck um einen durch die unvollständige Sättigung der Sandwich-Kammer bedingten Artefakt im Sinne einer Schwanzbildung (tailing) handelt. Diese Störung tritt auch häufig auf Chromatogrammen ohne Keilstreifen auf. Das Vorkommen von Lysolecithin in einer diesem Fleck entsprechenden Menge schließen wir deshalb sowohl im Ductus cochlearis als auch im Modiolus aus.

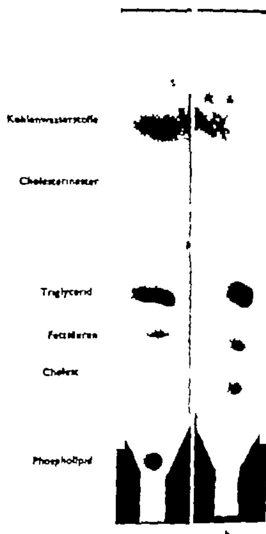


Abb. 1. Trennung der Neutralfette vom Ductus cochlearis (*a*) und einiger Vergleichssubstanzen (*b*): Tristearin, Stearinsäure und Distearin.

Die Lipide aus den Geweben des Ductus cochlearis und dem Modiolus

Beispiele für die Lipidspektren aus den oben genannten Schneckengeweben zeigen die Ab-

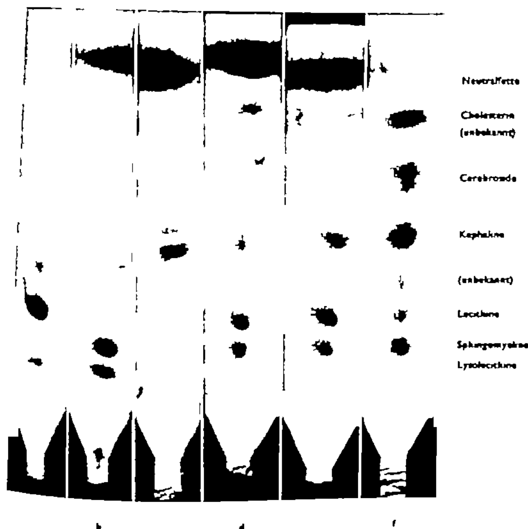


Abb. 2. Trennung der Phospho- und Glykolipide am Ductus cochlearis (d, e), vom Modiolus (f) und einiger Vergleichssubstanzen: (a) Lecithin, verunreinigt. (b) Sphingomyelin und Lysolecithin und (c) Kephalin, verunreinigt

bildungen 1 a und 2 d–f. Zur Identifizierung der einzelnen Flecke wurden einige Vergleichssubstanzen mit chromatographiert (Abb. 1 b, 2 a–c). Die Abb. 1 a zeigt, daß in den Geweben des Ductus cochlearis an Neutralfetten vor allem Cholesterin, freie Fettsäuren und Triglyceride vorkommen. In geringeren Mengen lassen sich auch Cholesterinester und wahrscheinlich Kohlenwasserstoffe nachweisen. Ein sicherer Nachweis von Diglyceriden gelang uns bisher nicht. Monoglyceride, die bei dem verwendeten Fließmittel zusammen mit den Phospholipiden am Startpunkt verbleiben, konnten mit einem anderen Fließmittel, welches Monoglyceride eluiert, nicht nachgewiesen werden (Esser 1969).

An Phospholipiden sind im Ductus cochlearis (Abb. 2 d) Kephaline, Lecithine und Sphingomyeline neben einer bisher nicht identifizierten Fraktion enthalten. Für diese bisher unbekannte Fraktion, die unterhalb des Cholesterins liegt, konnte die auf Grund des R_f-Wertes mögliche Identität mit Cardiolipin oder einem sog. „Kephalin B" (Wagner et al., 1961) bisher nicht bestätigt werden.

Für den Nachweis der einzelnen Lipidf

Windungen nicht so deutlich erkennbar die Fleckenintensität nimmt nach der bisher nur halbquantitativen Abschätzung, ebenso wie beim Cholesterin von der Schneckenspitze zur Schneckenbasis hin etwas zu.

Diskussion der Ergebnisse

Die Ergebnisse zeigen, daß die Dünnschicht Chromatographie in der hier beschriebenen *Form für die Untersuchung von Lipiden aus* Innenohrgeweben gut geeignet ist. Der Nachweis der Lipide des Ductus cochlearis die für die Innenohrfunktion von besonderem Interesse sind, erfordert relativ geringe Probemengen (etwa 1/4 Ductus cochlearis) Dadurch ist es möglich, von einem Tier die Gewebe des Ductus cochlearis sowohl auf Neutralfette als auch *auf Phospholipide durch mindestens je ein* Doppelchromatogramm zu untersuchen. Für die Untersuchung der Phospho- und Glykolipide des Modiolus sind die Bedingungen noch günstiger

Erste orientierende Untersuchungen an 10 Tieren gleichen Gewichtes ergaben keine qualitativen Unterschiede und, soweit nach halbquantitativer Abschätzung zu beurteilen ist, auch keine nennenswerten quantitativen Unter*schiede im individuellen Lipidspektrum* Genauere Angaben müssen quantitativen Bestimmungen vorbehalten bleiben

Der interessante Befund, daß die Lipidkonzentration in der Schnecke von der Basis zur Spitze hin zunimmt, steht im Einklang mit vielen histologischen und histochemischen Untersuchungen nach denen besonders die Hensenzellen der oberen Windungen einen hohen Lipidgehalt haben. Die hier vorliegende dünn*schicht-chromatographische Untersuchung er*gab, daß in den oberen Windungen vor allem die Neutralfette und zwar hauptsächlich die Triglyceride und Fettsäuren in größerer Menge vorhanden sind. Unter Berücksichtigung der histochemischen Befunde ist es wahrscheinlich, daß diese beiden Fraktionen den Hauptanteil der Lipide in den Hensenzellen stellen und daß die Fettsäuren überwiegend in ungesättigter Form vorliegen.

Eine unmittelbare Kombination von Histochemie und Dünnschicht-Chromatographie durch direktes Chromatographieren von Gewebeschnitten in Form der sog. „Histochromatographie (Curri et al., 1964 Breitenecker & Holczabek, 1966) erwies sich in Vorversuchen, offensichtlich wegen der zu geringen Lipidkonzentration der Innenohrgewebe als nicht anwendbar Wegen der großen Heterogenität der *Gewebe im Schneckenquerschnitt erscheint die*ses Vorgehen ohnehin als ungeeignet.

Zur funktionellen Bedeutung der einzelnen Lipidfraktionen können z. Zt. nur Vermutungen geäußert werden. Der überwiegende Teil der Lipide dürfte wohl als Strukturbestandteil, insbesondere der verschiedenen Membranen, wichtige Funktionen erfüllen Vielfältig diskutiert *wurde immer wieder der auffallend hohe Lipid*gehalt in den Hensenzellen. Aus der Speicherung in Tropfenform kann wohl geschlossen werden, daß es sich um Reservelipide handelt. Für die nutritive Funktion der Hensenzellen sprechen auch elektronenmikroskopische Befunde (Engström & Wersäll, 1958 u a.) Wir haben in einer früheren Arbeit (Gerhardt & Pieplow 1967) darauf hingewiesen, daß die Grundgeräuschbelastung des Ohres in der Na*tur im Frequenzbereich unter 1 kHz am größ*ten ist. Dementsprechend ist es durchaus sinnvoll, daß die Hensenzellen in dem Teil des Cortischen Organs, der offensichtlich akustisch am stärksten belastet wird, auch die größten Reserven aufweisen Schiff & Christiensen-Lou (1967) haben auf die Möglichkeit hingewiesen, daß die basalen Schneckenwindungen deshalb vulnerabler gegen akustische Traumen sind, weil die nutritive Versorgung über die Hensen*zellen hier schlechter ist. Andererseits* gibt es eine Reihe von Säugetieren die keine Fetttröpfchen in den Hensenzellen haben Die endgültige Klärung der Frage wird also funktionellen Untersuchungen vorbehalten bleiben müssen.

SUMMARY

Lipids obtained from guinea pig cochlea were investigated by thin layer chromatography using new

method of demonstration (Schindler & Wolf, 1968) In the tissues of the cochlear duct including the whole spiral ligament the following neutral lipids could be identified: cholesterol, free f tty acids and triglycerides. I addition, small amounts of cholesterol esters and carbohydrates were detected The following phospholipids were found in the same tissues: cephaline, lecithin, and sphingomyelin. A further fraction in the chromatogram has not yet been identified. Moreover the tissues of the modiolus contained cerebrosides as a main fraction along with another fraction as yet unidentified. The separate investigation of the single coils revealed that all lipid fractions found throughout the cochlear duct are lso present in each coil. The amount of neutral fats in the cochlea increases from basal towards apical coil. These findings are in agreement with histochemical investigations. The possible functional significance of these findings is discussed.

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PERIPHERAL FACIAL PALSY

A Clinical Material

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Abstract An unselected patient material of peripheral facial palsy from a known population group is presented. Out of 168 cases 144 (86%) were diagnosed as ischemic palsies of Bell' type. Bell's palsy strikes most patients in the beginning of middle-age and there is no difference in the frequency between men and women. Regardless of whether conservative or surgical therapy is initially used, an improved diagnosis *using electro-physiological recordings ought to* be strived for. Conservative therapy with Rheomacrodex®—a colloid osmotic hypertonic dextran solution with anti-sludge effect—and histamine intravenously has not proven to be better than using only vasodilators. Patients having an objectively provable axon-injury ought to be operated on within weeks in accordance with experience recounted in an additional article (Lagerholm et al., 1971)

pheral facial palsy has long been the cause great differential diagnostic and therapeutic problems. The pathophysiology of spontaneous "ischemic facial palsy" is still largely unknown, which has led to variations in methods of examination and treatment. Unselected and uniformly treated patient materials are few. The relatively good prognosis with a high percentage of spontaneous regression often leads to varied treatment of the symptoms in out-patient clinics with no thorough investigation or follow-up of the individual case. It is therefore difficult to evaluate correctly the results of different forms of conservative and operative treatment.

At our hospital, which is the only one in a town with about 250 000 inhabitants we have the advantage of seeing most of these patients in the ENT department at an early stage of the disease for primary investigation and early treatment in co-operation with other clinics and practitioners of the city. The purpose of this article is to describe the frequency of peripheral facial palsy in different age-groups in a known population and to report the results of *a prospective investigation regarding a uniform* therapy of Bell's palsy during a 5 year period.

MATERIAL AND METHODS

During the years 1963–67 168 cases with different types of peripheral facial palsy have been given a short hospital care at our clinic in order to get a thorough examination. Of these 164 have been investigated according to a fixed scheme. In cases with complete palsy the neuro-muscular function of the facial nerves has been investigated using different electro-*diagnostic methods on the 4th, 7th and 11th* day after the palsy's debut (Lagerholm et al. 1971)

In Fig. 1 the distribution of all the cases of palsy with different etiology is shown. The largest group, comprising 144 patients, had "ischemic palsy" of Bell's type. Four cases were due to fractures through the temporal bone with the facial canals being engaged. Three of these were operated on for decompression of the nerve. The fourth was a case of post traumatic palsy which receded after conservative treatment. In 3 cases the palsy was caused by otitis media. Two of these con-

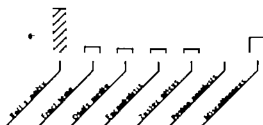


Fig 1 Investigational material distributed according to etiology □ conservative therapy ▨ conservative therapy + decompression.

sisted of acute otitis with a rapid regression of the palsy after antibiotic therapy while the third case—in a woman who had had chronic otitis for 50 years—was a cholesteatoma, which penetrated to the facial canal at the level of the lateral semicircular canal. Three patients had virus meningo-encephalitis. Three typical herpes zoster oticus cases are also included in the material One case of chronic otitis had postoperative palsy after mastoidectomy so that reoperation and decompression of the facial nerve became necessary In two further ear-operations, one for congenital atresia of the outer and middle ear and one for a large cholesteatoma, partial palsy followed due to postoperative oedema. A man included in the mixed group given in Fig. 1 had had a radical operation in the right ear 20 years earlier and had become temporarily palsied during cleansing of the radical cavity A patient who 36 years earlier had been operated on for chronic otitis, with operative trauma of the facial nerve, has also been reoperated with resection of scar tissue and end-to-end nerve suture EMG recordings have shown that this patient has later shown signs of reinnervation and improved movement of the corresponding muscle-groups despite the long period of latency (Lagerholm et al. 1971) A case of protracted external otitis gave temporary palsy which lasted for 3–4 weeks. One case of peripheral facial palsy which made its debut in the lower branch and for a period of 1 week caused total peripheral palsy in all the branches, proved to be a case of cancer of the parotid gland In another patient, the trunk of the facial nerve was cut off by accidental external trauma in the region of the parotid gland This function returned after surgical intervention and nerve suture Finally a pregnant woman, who is included in the material developed lesio auris interna with total deafness, reduced caloric reactions and after a further few months a debut of facial palsy which, however regressed during the course of half a year

Of 144 cases with Bell's palsy 74 were women and 70 men—most of them at the beginning of middle-age (Fig. 2) Nine patients had earlier had unilateral palsy and 3 patients had had bilateral palsy One of these patients, a 47 year-old woman, also had two sisters with facial palsy in their case histories, which induced thoughts of Melkersson's syndrome The other criteria of facial oedema and lingua plicata were lacking, however Five cases of Bell's palsy occurred in the final stage of pregnancy and 6 patients had a day or so prior to the debut of the palsy received dental treatment on the side where the palsy appeared.

During the actual period standard treatment for ischemic facial palsy has been Rheomacrodex* intravenously with the addition of histamine to give maximum capillary dilatation. The patients received infusion twice daily in hospital (2 × 250 ml Rheomacrodex with 2 mg histamine) for about 1 week and thereafter capillary dilatives *per os*.

* A colloid osmotic hypertonic dextran

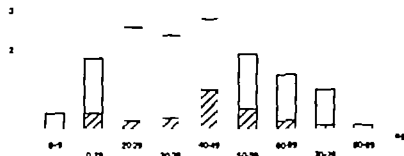


Fig 2 Investigational material distributed according to age. □ conservative therapy; conservative therapy + decompression.

RESULTS

Of the 144 patients with Bell s palsy 103 were able to complete the course of treatment (Fig. 3) In 13 cases it had to be broken off due to side-effects in the form of headache and falling blood-pressure The other patients, who for some reason or other did not receive Rheomacrodex histamine treatment, were usually given vasodilators such as Complamin[*] with a dosage of 3 × 0.3 g. Patients who have shown no or only slight signs of regression during this conservative therapy have had the facial nerve surgically decompressed in its lower tratemporal course The operation was most often made after 3 weeks.

Using the treatment described above 103 patients with Bell s palsy that is 72 per cent of the total group have thus been completely restored with normal functioning of the facial nerve (Fig. 4) 27 patients have undergone surgery Of these only six have had full regression, while 14 have had partial restitution of the facial nerve function but this was, however with a satisfactory cosmetic result. Seven of the operated cases have for different reasons, not been available for regular check-ups following surgery but they have been sent a questionnaire This indicated that in these cases too there is a certain amount of functional reduction, though not of any practical importance. No case of remaining total palsy is included in the material If the patients who received Rheomacrodex histamine are compared with those given Complamin or other remedies, one finds no certain improvement in the treatment results, however Thus about

* Nicotinic acid purine component.

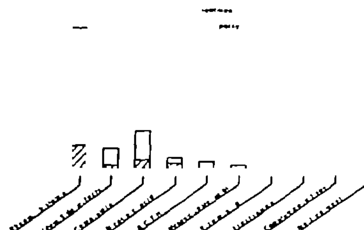


Fig 3 Investigational material distributed according to form of therapy conservative therapy; conservative therapy + decompression.



Fig 4 Investigational material distributed according to recovery time. □ conservative therapy ▨ conservative therapy + decompression.

50 per cent had total regression within 6 weeks in both groups and a further 20 per cent within 4 months. The number of operated cases is, however percentually somewhat less in the Rheomacrodex-group than in the other one namely 17 per cent and 21 per cent respectively. Engagement of chorda tympani was tested by examination of all four taste qualities. The result can be seen in Fig. 5

DISCUSSION

If one compares the current material with, for example, Cawthorne's investigation from 1952, one finds certain differences concerning the etiology Cawthorne namely indicates 69 per cent of the palsy cases as having ischemic genesis, whereas we have had 86 per cent in our material. Bearing in mind that this material is unselected and reflects the disease panorama in an exclusive population group the latter figure would seem to be the most plausible one.

Ischemic facial palsy is found in all age-groups, but is most common between the ages of 20 and 50 The syndrome thus strikes in the first place patients in their most active years. We have found no difference in distribution between the sexes, so that it seems probable that there are not any significant constitutional or hormone factors affecting the development of this type of facial palsy There has been much discussion about the importance of cold exposure in the genesis of ischemic palsy In our material only about 50 per cent have been exposed to cold or draught in direct connexion with the debut of the disease which would hardly support this theory

It is of especial interest for the otologist to check the function of the chorda tympani. This is done by examining the sense of taste and in the material we find 60 per cent having a reduction of taste We have, however not found any substantiation for the assumption that an engagement of the chorda tympani in Bell's palsy is an unfavourable prognostic sign, as we have not had a higher percentage of cases requiring surgical decompression in this group than in the group with normal sense of taste (Fig. 5)

Treatment of Bell's palsy has earlier varied a great deal but during the last few years the majority of methods have been based on improving the blood supply to the facial nerve in canalis Fallopii or on reducing the initial oedema due to selfcompression in the nerve

Capillary dilatives as well as diuretics and cortisone preparations have been used to combat ischemia and oedema. When penetrating the literature in this field, it is difficult to judge the effects of the different therapeutic measures, partly due to the spontaneous tend-

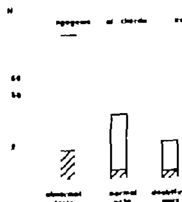


Fig 5 Investigational material distributed accor... to engagement of the chorda tympani. □ ... tive therapy ▨ conservative ... sion.

ency to heal (Peitersen & Andersen, 1967) and partly because of the lack of uniform treatment methods over a longer period of time For many samples of patients the figure 70–85 per cent is given for total regression regardless of the method of treatment. Most of the patients in our material have since 1963 been consistently treated with Rheomacrodex histamine. This form of treatment was based on a rather large group of theoretical and clinical investigations regarding the so-called sludge-phenomenon, which showed that aggregation of red blood-corpuscles and accompanying circulatory disturbances could be reduced and the capillary-flow improved by infusion of Rheomacrodex with an average molecular weight of about 40 000 (Gelin & Ingelmann, 1961) Unfortunately it was shown after the 5 year period had ended that this form of treatment in ischemic facial palsy is not superior to methods practised earlier The investigation does not, then, confirm the assumption that microcirculatory disturbances in Bell's palsy are to any great extent caused by sludging of red blood corpuscles in the capillaries of the nerve

Taverner (1966) has earlier tried different capillary-dilating or oedema-reducing forms of therapy and later described good results with TH. That therapy should also give better ts than cortisone-treatment, which was earlier In our clinic we have now changed the conservative therapy to ACTH, but it is still too early to state anything about its effect It is expected that these trials will be completed under a new 5 year period.

A total of 20 per cent of the cases of palsy in this material were judged to be unsuitable for conservative therapy so that surgical decompression had to be considered. This figure is in approximate agreement with those given for other materials It can, however be pointed out that only 6 of 27 operated on have been completely functionally restored. Among other things, this ought to be due to the long latency period prior to operation. If the compressed nerve is to recover it is probable that the decompression ought to be performed much sooner We have in consultation with neurophysiological experts drawn the conclusion that the decision ought to be made not later than on the 11th to 14th day when the third EMG-result is available (Lagerholm et al., 1971). This is also in agreement with the opinion of other authors (Jongkees 1969 Hiestand et al., 1969)

As acute facial palsy often implies severe physical and psychical trauma for the patient, we consider it warranted to give a standardized treatment in hospital during the first week. Besides, we believe that patients with such conspicuous symptoms are in need of an initial care and special attention. The hospital stay also enables improved individual diagnosis according to a standardized system of examinations. We agree with May (1970) in his statement that such a system is imperative for a correct evaluation of clinical and therapeutical experiences.

ZUSAMMENFASSUNG

Beschreibung eines unselektierten Patientengutes von peripheren Gesichtslähmungen, das aus einer bekannten Bevölkerungsgruppe stammt. Von 168 Fällen wurden 144 (86 v.H.) als ischämische, d. h Bell'sche Lähmungen diagnostiziert. Die Bell'sche Lähmung trifft meistens Patienten mittleren Alters und es besteht in dieser Hinsicht kein Unterschied zwischen den Geschlechtern. Abgesehen davon, ob anfangs konservativ oder chirurgisch vorgegangen wird, sollte eine verbesserte Diagnose und der Gebrauch elektrophysiologischer Registrierung angestrebt werden. Konservative Therapie mit Rheomacrodex®—eine kolloidalen, osmotisch hypertonen Dextranlösung mit anti-sludge Effekt auf rote Blutkörperchen—und Histamin i hat sich gegenüber dem Gebrauch von nur gefässerweiternden Pharmaka nicht als vorteilhaft erwiesen. Patienten mit objektiv nachweisbaren Axonbeschädigungen sollten innerhalb von zwei Wochen operiert werden, entsprechend eigener Erfahrungen, die in einem kommenden Artikel (Lagerholm et al., 1971) demnächst vorgelegt werden.

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NASOPHARYNGEAL FIBROMA WITH EXTRAPHARYNGEAL EXTENSIONS

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Abstract Five cases of multiple extrapharyngeal extension of nasopharyngeal fibroma seen during the last 8 years were operated by a combined transzygomatic and transpalatal/or per via naturalis approach. The transzygomatic approach is excellent for exposing the lateral extension of this tumour and for efficient control of bleeding under direct vision. Salient features of its clinical behaviour operative findings and pitfalls in the management are described.

Very few instances of extrapharyngeal extension of the nasopharyngeal fibroma have been reported in the literature. Amongst the recent eports, Sardana (1965) described 4 cases of ek extension, Samy & Girgis (1965) re- rted 3 cases of lateral extension from Egypt where the incidence of nasopharyngeal fibroma is approximately one case per 50 000 ear nose and throat patients. More recently Bhatia et al. (1967) described 17 cases of lateral extension of this tumour from a total series of 92 cases studied over a period of 27 years. With such a low incidence of these extrapharyngeal extensions, it is not surprising that there is a considerable diversity of opinion not only as regards the ideal surgical approach to these tumours, but also about their pathway of extension.

MATERIAL AND METHODS

Only 5 cases out of a total of 21 cases of nasopharyngeal fibroma seen by the author during the last 8 years had extrapharyngeal extension of this tumour All 5 cases were treated by a combined transzygomatic and transpalatal or per via naturalis approach for the removal of parent tumour in the nasopharynx and its extrapharyngeal extensions.

OPERATIVE TECHNIQUE

The essential steps of the transzygomatic approach consist of a curved 4 cm long horizontal incision over the arch of zygoma about 1 5 cm in front of the tragus and extending towards the outer canthus of the eye. Another vertical incision starting from the mid-point of the horizontal incision and extending towards the angle of the mouth for about 3 cm is optional for better exposure of massive lateral extensions of the tumour in the cheek, maxillary antrum and retromandibular region. The temporalis fascia is divided along the upper border of the zygomatic arch and the latter is cut and reflected downwards with the attached fibres of the masseter muscle. The fibres of the temporalis and the pterygoid muscles over the tumour mass are also divided and retracted. The maxillary artery and its branches are ligated and divided (Fig. 1). The lateral extensions of the tumour are then separated and delivered into the wound (Fig. 2). The parent tumour in the nasopharynx and the nasal fossa is also separated by transpalatal or per via naturalis approach. The entire tumour

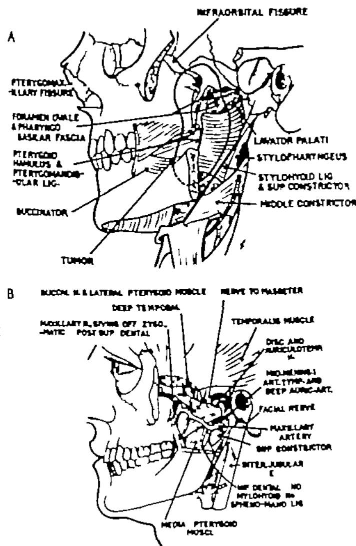


Fig. 1. (*A*) Surgical anatomy of the infratemporal region showing the pathway of the lateral extension of nasopharyngeal fibroma. (*B*) Important anatomical structures that are encountered in the exposure of the tumour by the transzygomatic approach.

then can be removed in one piece by pulling the extrapharyngeal extension of the tumour from outside or in two pieces by dividing the pedicle at the exit of the tumour from the nasopharynx at the upper border of the superior constrictor muscle.

CASE REPORTS

Case 1

M. L. a 12 year-old boy was first admitted on August 8 1963 with complaints of left nasal obstruction and recurrent bleeding from the nose and mouth for 1 $^1/_2$ years. A large lobulated nasopharyngeal fibroma extending into the sphenoidal sinus was removed by transpalatal route after a preliminary left external carotid ligation. The patient returned on November 14 1966 with recurrence in the nasopharynx, mild proptosis and massive extension of the tumour in the left cheek and temporal region (Fig. 2). A short course of deep X-ray therapy of 1 200 R was given to reduce the vascularity of the tumour and 2 months later it was removed by the combined transzygomatic/per via naturalis approach. The lateral









Fig 2 (*A*) Swelling of the cheek and temporal region (case 1) due to lateral extension of the tumour (*B*) Transzygomatic exposure of the lateral extension. (*C*) Postoperative photograph. (*D*) Tumour specimen showing various extensions.

extension of the tumour in the left temporal fossa, antrum, cheek and retromandibular region was connected with the parent tumour in the nasopharynx by a pedicle passing between the upper border of the superior constrictor muscle and the base of the skull The tumour had partially destroyed the posterolateral wall of the maxillary antrum, pushing up its orbital plate, coronoid process of the mandible and the pterygoid plates from behind, forwards The patient is now free from recurrence (Fig. 2)



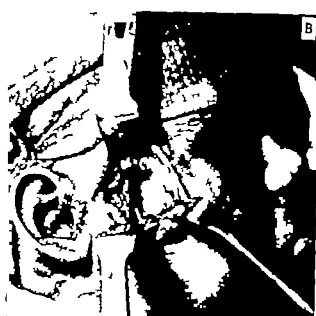




Fig 4 (*A*) Swelling of the right cheek (case 3) and nasal obstruction are noticeable. (*B*) Transzygomatic exposure of the lateral xtension. (*C*) Wound after removal of the tumour

Case 2

S. P an 11 year-old boy was seen on October 16 1967 with complaints of nasal obstruction, recurrent epistaxis and swelling of the right side of face of about 2 years duration (Fig. 3) The nasopharyngeal fibroma (biopsy proved) was filling the right nasal fossa completely and pushing the nasal septum to the opposite side Skiagram showed enlargement of the infratemporal region on the right side. After a short preoperative course of deep X-ray therapy (1 000 R) the nasopharyngeal tumour and its extrapharyngeal extensions were removed completely in two parts, by a combined transzygomatic/transpalatal approach. There was an ex tension of the tumour in the right maxillary antrum through an erosion of its postero-lateral wall. There was no destruction of the pterygoid plates. The tumour was found to be coming out of the nasopharynx above the upper border of the superior constrictor muscle Patient is now free from recurrence (Fig. 3).

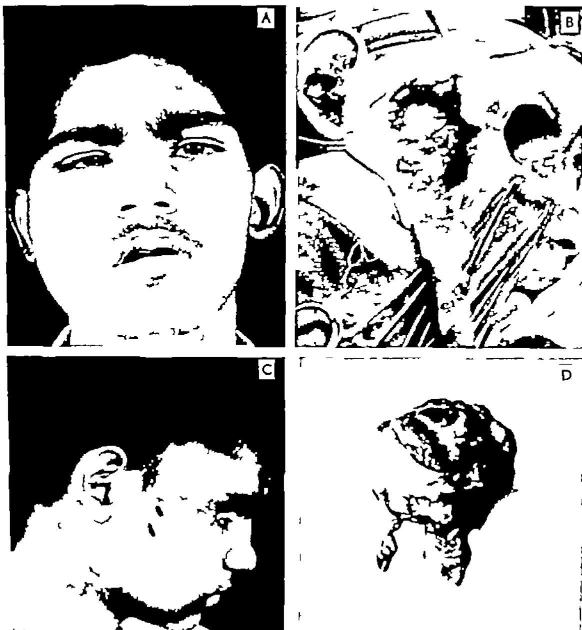


Fig 5 (*A*) Preoperative photograph (case 4) showing swelling of the right cheek. (*B*) Photograph during second operation. The orbit has been exenterated Tumour recurrence is visible in the wound. (*C*) Patient after orbital exenteration. (*D*) Eyeball with tumour mass attached to its posterior aspect.

Case 3

J., a 14-year-old boy of very weak build, was admitted on January 31 1968 with swelling of the right side of face, nasal obstruction, and occasional bouts of unprovoked epistaxis of 3 years duration (Fig. 4) An unsuccessful attempt at removal of the swelling in the cheek was made 1 /₂ years ago in another hospital by an incision along the lower border of the mandible. Clinical examination and biopsy revealed nasopharyngeal fibroma. Radiological examination showed profound enlargemen



Fig 6 (*A*) Case 5 before operation showing swelling of the right cheek and nasal obstruction. (*B*) Lateral extension of the tumour delivered in the wound. (*C*) Postoperative fistula which healed later (*D*) Tumour specimen with its extensions.

the right infratemporal region. A preoperative short course of deep X ray therapy was given and about a month later 325 ml of blood was transfused 1 day prior to surgery because of his anaemic condition. However he developed haemolytic jaundice immediately after blood transfusion and operation had to be postponed for 2 weeks During this period he had further bouts of severe epistaxis. A fresh sample of patient's blood could not be matched with any donor and so it was decided to operate with the help of plasma. The transzygomatic incision revealed a small extrapharyngeal extension of the tumour which was excised by di-

viding the pedicle connecting it to the main mass in the nasopharynx. The latter was removed by transpalatal incision, which revealed a broad-based tumour extending into the sphenoidal sinus, both nasal fossae and right maxillary antrum. The repair of the transpalatal incision was just started when sudden cardiac arrest occurred. All attempts to revive the patient by open cardiac massage failed. An exploration of the wound revealed partial destruction of the pterygoid plates with intact pterygomaxillary fissure.

Case 4

R., an 18-year-old boy was admitted on December 4 1968 with complaints of right nasal obstruction with recurrent epistaxis and swelling of the cheek (Fig. 5) of about 10 months duration. Clinical examination and biopsy revealed nasopharyngeal fibroma pushing the right half of soft palate down and filling the right nasal fossa completely. Skiagram of the skull showed osteoporosis of the right coronoid process which was pushed outwards. The patient was sent for a short course of deep X ray therapy but was given instead a full course of 5 000 R during 5 weeks. A combined transzygomatic-transpalatal approach revealed the extrapharyngeal portion of the tumour on the superior constrictor muscle pushing the coronoid process of the mandible outwards and connected by a pedicle to the nasopharyngeal portion over the upper border of the superior constrictor muscle. There was no erosion of the pterygoid plates. The tumour was also extending into the sphenoidal sinus. It could only be removed in pieces as it had become very friable due to full course of preoperative deep X-ray therapy. The patient returned 3 months later with recurrence in the cheek, sphenoidal sinus, nasopharynx, and orbit, and was again operated upon by the combined approach (Fig. 5). The orbit had to be exenterated as the tumour was extending into it through the inferior orbital fissure. The patient remained well for about 2 months but again developed recurrence in the nasopharynx and sphenoidal sinus. Fundus examination of the left eye revealed optic atrophy. The patient did not agree to another surgical intervention and died in September 1969 presumably due to intracranial extension through the spenoidal sinus.

Case 5

B R., an 11 year-old boy was admitted on January 9 1969 with complaints of right sided nasal obstruction, frequent epistaxis, and swelling of the right side of face of about 2 months duration (Fig. 6). Clinical examination and skiagram revealed nasopharyngeal fibroma with extension into right infratemporal region. The patient was given a preliminary short course of deep X-ray therapy (2 000 R). The tumour was removed a month later by the combined approach. The extrapharyngeal extension in the cheek and temporal fossa was found to be connected to the nasopharyngeal portion by a pedicle passing between the upper border of the superior constrictor muscle and the base of the skull. There was partial destruction of the pterygoid plates from behind, forwards. The nasopharyngeal portion showed extensions into the right nasal fossa and sphenoidal sinus. The patient is now free from recurrence.

DISCUSSION

All the cases in the present series had multiple extrapharyngeal extensions in other regions in addition to cheek extension. Extension into the sphenoidal sinus occurred in 4 cases, maxillary antrum 3 cases, temporal fossa 2 cases and orbit 1 case. Handoussa et al (1954) also reported 4 cases with combined cheek and orbital extensions.

Many workers in the past (*Friedberg*, 1[illegible] Erich, 1955 Tapia *Acosta*, 1956 [illegible] 1959) have described the lateral [illegible] this tumour through *pterygo-maxillary* [illegible] Samy & Girgis (1965) [illegible] only hypothetical and lacked [illegible] proof. They argued that such [illegible] large lobulations can hardly [illegible] through such a narrow fissure [illegible]

on the basis of autopsy findings that this tumour extends laterally through the pharyngobasilar fascia in the weak part of the pharyngeal wall between the upper border of the superior constrictor muscle and the base of the skull (Fig. 1) Once on the surface of the superior constrictor muscle, the tumour extends downwards and forwards across the pterygo-mandibular ligament on the buccinator muscle to the cheek and may also extend upwards into the temporal fossa. During the exit, destruction of the pterygoid plates may occur widening the outlet of the tumour These observations of Samy & Girgis (1965) have been confirmed without exception radiologically and during operation in the present series by the following findings (*a*) Passage of the pedicle connecting the cheek extension with the nasopharyngeal portion between the upper border of the superior constrictor muscle and the base of the skull in all the 5 cases. (*b*) Partial destruction of pterygoid plates from behind, forwards and intact pterygo-maxillary fissure, in 3 cases. (*c*) Intact pterygoid plates in 2 cases.

Martin (1954) described an alternative route to the cheek by anterior extension of the into the nasal fossa and then by transmaxillary extension to the cheek, destroying the medial and anterolateral wall of the antrum. Bhatia et al (1967) also reported 3 cases of transmaxillary extension to the cheek. In the present series extension of the tumour into the maxillary antrum occurred through erosion of its posterolateral wall by the cheek extension. There was no destruction of antro-nasal wall in these cases by the tumour mass in the nose

Several surgical approaches to the cheek extension have been described in the literature. Härmä (1959) quoted Kremer (1953) advising pre auricular incision and cutting the ramus of the mandible. Rao (1961) split the upper lip and cutting through the naso-labial furrow reflected a large cheek flap Sardana (1965) advised a separate sublabial incision. Bhatia et al. (1967) advocated forward extension of the transpalatal incision into the sublabial plane curving round the maxillary tuberosity A glance at the surgical anatomy of the infratemporal region (Fig. 1) will immediately suggest that all these approaches are blind as they do not expose the tumour at its exit from the nasopharynx and can cause serious bleeding from the pterygoid plexus of veins, branches of the maxillary artery and the embryonic blood vessels of the tumour mass which is likely to be left behind by a blind procedure. In fact Misra & Bhatia (1964) have mentioned postoperative haematoma of the cheek in their cases Moreover parts of the tumour lying under the masseter muscle and in the temporal fossa cannot be removed satisfactorily by any of these approaches The transzygomatic approach for the cheek extension first described by Samy & Girgis (1965) is an ideal approach for these extensions from all these points of view and provides adequate exposure of the tumour and efficient control of the bleeding.

Recurrence of the nasopharyngeal fibroma is mostly due to incomplete removal of the growth. This was probably the cause in the first case, and was certainly so in case 4 where the tumour had become very friable due to a full course of preoperative deep X-ray therapy Blood transfusion during operation is imperative and operation should not be done if blood is not available, for any reason. The chances of cardiac arrest during surgery increase if blood loss is not replaced by rapid transfusion.

ZUSAMMENFASSUNG

Fünf Fälle von vielfachen extra-pharyngealen Ausdehnungen von „nasopharyngeal fibroma" wurden in den letzten 8 Jahren durch einen kombinierten transzygomatischen, einen trans-palatalen oder durch einen per via naturalis Zugang operiert. Der trans-zygomatische Zugang ist bestens geeignet für die Enthüllung der seitlichen Ausdehnung des Tumors und für die wirksame Kontrolle der Blutung in direkter Sicht Beschrieben sind die hervorragenden Aspekte seines klinischen Verhaltens, der operativen Feststellungen und Irrtümer der Handhabung.

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ELECTROPHYSIOLOGICAL PROPERTIES OF LARYNGEAL REFLEX CLOSURE

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Abstract Reflex responses were elicited in the recurrent laryngeal nerve by electrical stimulation of any sensory nerve trunk in the laryngo-pharyngeal region. Electrical stimulation of the internal branch of the superior laryngeal nerve produced. (1) Reflex response in each laryngeal muscle including the posterior cricoarytenoid. (2) Abduction of the vocal cord after the adductors had been denervated. (3) Inhibition of activity in fibers f the motor nerve to the posterior cricoarytenoid which discharged dur ing inspiration. (4) Consistent EMG responses in adductor muscles to stimulation of the internal branch of the superior laryngeal nerve at frequencies over 10/sec. The posterior cricoarytenoid muscle re sponded only to the first few stimuli at these frequencies and was then inhibited completely (5) More intense response during expiration in the adductor muscles and during inspiration in the abductor muscle. The threshold, latent period and duration of the reflex response differed for each muscle and for each f ber in their motor nerves.

Protection of the lower airways against intrusion of foreign matter is the most primitive and most important function of the larynx. The glottis is reflexly closed on touch stimulation of the laryngeal mucosa or by electrical stimu lation of the internal branch of the superior laryngeal nerve (SLN) Several reports on protective closure of the larynx state that reflex responses occur in each laryngeal muscle the adductor muscles contracting reflexly and the abductor muscle being reflexly inhibited dur ing inspiration (Doty & Bosma, 1956 Mårtensson, 1963 Kirchner & Suzuki, 1968 Suzuki & Kirchner 1969) Mårtensson reported that ipsilateral stimulation of the internal branch of the SLN in the dog elicited reflex discharges in all of the adductors but never in the abductor The latter showed only reflex inhibition of its inspiratory activity and the inter arytenoid only a feeble response with repetitive stimulation. Suzuki and Kirchner studied an electroneurogram of a small filament of the recurrent laryngeal nerve (RLN) in the cat and reached the same conclusions, namely that the posterior cricoarytenoid muscle (PCA) is reflexly inhibited.

The idea of a reciprocal mechanism between the adductors and the abductor is attractive, but probably not complete. It is true that all of the nerve fibers which are active during inspiration innervate the PCA muscle, but it may also be true that the PCA muscle is not inner vated exclusively by such fibers. In this re gard, some investigators reported that reflex activation was elicited not only in the adduc tors but in the abductor (Yamashita & Urabe, 1959) For complete delineation of how each laryngeal muscle responds to afferent stimula tion, recordings of reflex responses must be made from filaments in the nerve supplying each individual muscle

Our objective in the present study was an electrophysiologic delineation of the laryngeal reflex pathways in the protective closure of the larynx.

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ANATOMY

Detailed studies of the motor supply of each laryngeal muscle formed a preliminary but essential part of the present study The RLN in the cat enters the larynx just under the level of the cricopharyngeus muscle and just posterior to the crico-thyroid joint. Before entering the larynx, a large branch, the ramus communicans, runs upward behind the PCA muscle and joins the internal branch of the SLN It is probably composed mainly of afferents from the thorax.

The main trunk of the RLN runs upward along the anterior and lateral border of the PCA muscle and gives off a small branch posteriorly which enters and innervates the PCA muscle. Just above this twig, another small branch leaves the main trunk and enters the space between the PCA muscle and the cricoid cartilage. It then enters and innervates the interarytenoid muscle A "posterior" branch of the RLN sometimes credited with innervating the interarytenoid muscle was not found in our experiments (Hollinshead, 1954 Lemere, 1932 Vogel, 1952)

At the level of the lateral cricoarytenoid muscle a small twig leaves the main trunk, runs anteriorly and innervates this muscle. The main trunk continues upward and branches into two small twigs which innervate the thyroarytenoid muscle. One trunk runs along the inferior edge of this muscle and enters its anterior part. The other branch enters the posterior part of the same muscle. In some cats, a very small twig leaves the main trunk of the RLN at the same level, runs posteriorly and unites with the internal branch of the SLN This probably represents a group of afferent fibers from the larynx and thorax. The motor supply to each muscle is schematically shown in Fig. 1

METHODS

Twenty-six adult cats were utilized in this series of experiments. Each cat was anesthetized with intraperitoneal injection of 30 mg/kg of sodium pentobarbital (Nembutal) Observation of the reflex responses was made two hours after the injection and the depth of anesthesia at that time was Stage III, plane 1 The larynx and trachea were exposed by a vertical midline incision. Tracheotomy was done low in the neck and a vinyl canula inserted. The right RLN was cut in the neck together with the SLN of the same side When recordings were made from the nerve, the cat was paralyzed with an intravenous injection of gallamine triethiodide (Flaxedil)

1 *Reflex responses in the RLN by stimulation of afferents in the laryngo-pharyngeal region*

The left RLN was cut in the neck and its central cut end was placed on platinum wire recording electrodes. Ipsilateral nerve trunks (lingual, hypoglossal, glossopharyngeal, pharyngeal, both internal and external branches of the SLN and the RLN itself) were separated from surrounding tissues and the central cut end of each was placed on stimulation electrodes. Repeated single shock stimulations were furnished by a Grass S-4 stimulator Reflex responses in the RLN were observed by oscilloscope (Tektronix 564) after amplification with Tektronix RM122, monitored by audiosystem (Grass AM5) A Grass camera then recorded from a second oscilloscope.

2. *Reflex responses of each laryngeal muscle*

The reflex response in the cricothyroid muscle has already been reported in a previous study (Murakami & Kirchner 1970) The remaining intrinsic laryngeal muscles were studied and are being reported here After the inferior pharyngeal constrictor muscles had been detached and the mucosal lining of the pyriform sinus retracted, the thyroid w
cut in the midline and retracted
cricothyroid muscle. Each
and its nerve were thus
cording. The surgical

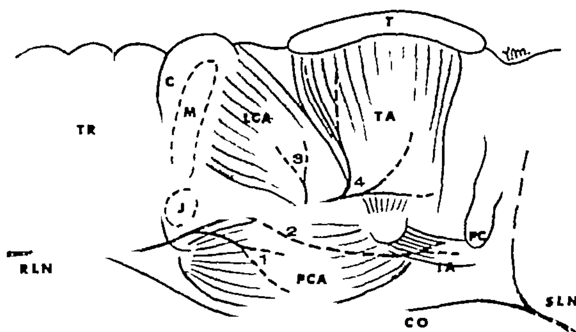


Fig 1 Innervation of each laryngeal muscle.
T thyroid cartilage (cut edge)
C cricoid cartilage
M cricothyroid muscle attachment
J cricothyroid joint
TR trachea
TA thyroarytenoid muscle
LCA lateral cricoarytenoid muscle
IA interarytenoid muscle
PCA posterior cricoarytenoid muscle
PC posterior commissure
1 nerve innervating PCA muscle
2 nerve innervating IA muscle
3 nerve innervating LCA muscle
4 nerves innervating TA muscle
RLN recurrent laryngeal nerve
SLN superior laryngeal nerve (internal branch)
CO ramus communicans

ral oil The central cut end of the internal branch of the left SLN was placed on the stimulation electrodes. Reflex responses in each muscle were recorded by concentric bipolar needle electrode and a specially made spiral copper-wire electrode Recording of the response in one muscle was carried out after denervation of other muscles to prevent artifacts occasioned by current spread The nerve twig to each muscle was carefully separated from surrounding tissue and placed on the recording electrode. To prepare a sufficient length of nerve for recording, it was sometimes necessary to separate the nerve deep into its muscle, tearing away some of the muscle fibers. For exposure of the nerve to the interarytenoid muscle, it was always necessary to dissect away the PCA muscle

RESULTS

1 *Reflex responses in the RLN to stimulation of afferents in laryngopharyngeal region*

The main trunk of the lingual nerve proximal to the branches supplying the faucial and oral mucosa was cut at the level of the styloglossus muscle and prepared on the stimulation electrodes. Every stimulus to the nerve elicited a reflex response in the RLN (Fig. 2 A) The threshold for this reflex response was extremely high, about 10 times higher than that for the internal branch of the SLN and the response appears most likely to be part of the pain reaction.

The hypoglossal nerve is primarily motor although afferents have been reported (Blom, 1960 Cooper 1954 Nakamura, 1968 Sauer

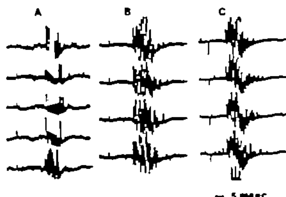


Fig. 2. Reflex responses in the left RLN to stimulation of (A) the ipsilateral lingual nerve, (B) hypoglossal nerve (C) glossopharyngeal nerve. Latent period and duration of the response are characteristic for each nerve. The threshold for response to stimulation of the glossopharyngeal nerve is about three times higher than for the internal branch of the SLN that of the lingual and the hypoglossal nerves are about ten times higher Tracings were made in the same cat. Differences in their patterns are due mainly to difference in electrode-distance.

land & Mizuno 1968 Tarkan & Abou-El-Naga, 1947) Sauerland & Mizuno recorded a reflex response in the RLN to stimulation of the main trunk of the hypoglossal nerve. In the present study every stimulus to the main trunk of the hypoglossal nerve elicited distinct reflex responses in the RLN (Fig. 2 B) The threshold for this reflex was almost the same as that for the lingual nerve, about 10 times higher than for the internal branch of the SLN

The glossopharyngeal nerve, on the other hand, is composed of many sensory fibers to the pharynx and tongue. Since the main trunk of the nerve was too short to allow its being placed across stimulation electrodes, a small branch which enters the mucosa of the lateral wall of the pharynx was separated just under the mucosa and placed on the electrodes. Brisk responses were reflexly elicited in the RLN with every stimulus of this small branch (Fig. 2 C) The threshold for the reflex was about three times higher than that for the internal branch of the SLN

The pharyngeal nerve is usually described as motor to the pharyngeal constrictor muscles and to the musculature of the upper cervical esophagus (Hwang et al., 1948 Miller et al., 1964) As we have already reported the nerve is likely to respond to laryngeal stimulation, showing reflex inhibition in the activity in the fibers to the middle pharyngeal constrictor or to the thyropharyngeus muscle Reflex activation occurred in the fibers to the cricopharyngeus muscle (Murakami & Kirchner 1970) On the other hand, there is no report of afferents in the pharyngeal nerve. Nevertheless, even in the cat whose SLN together with the glossopharyngeal nerve had been cut bilaterally reflex closure of the larynx was elicited on touch stimulation of the mucosa of the pyriform sinus. The result indicates that the pharyngeal nerve most likely contains some afferent fibers from parts of the pyriform sinus. Further electrical stimulation of the central cut end of the pharyngeal nerve elicited a reflex response in the RLN in every instance (Fig. 3 A)

In addition, then, to the usual nerves some afferent pathways for the laryngeal reflex exist in the lingual nerve and in the main trunk of the hypoglossal. However whether these pathways participate in the laryngeal protective reflex is questionable because of their extremely

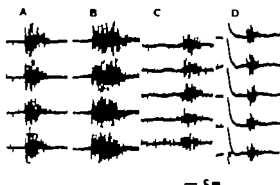


Fig. 3. Reflex responses in the left RLN to stimulation of the (A) ipsilateral pharyngeal nerve, (B) internal, (C) external branch of the SLN and (D) central of the same RLN Latent period and duration of the response are characteristic of each. Threshold for the reflex is about three times higher for the pharyngeal nerve and ten to twenty times higher for the external branch of the SLN and for the RLN than that for the internal branch of the SLN In C and B the responses are few and of low voltage.

high thresholds. They serve, possibly a state of emergency Afferents in the glossopharyngeal and pharyngeal nerves, on the other hand, are widely distributed in the pharyngeal mucosa and excited the reflex circuit even at low voltages. These nerves, for this reason, probably share in the protective mechanism of the larynx.

Stimulation of the internal or the external branches of the SLN and of the RLN itself evoke reflex responses in the RLN as previously reported (Suzuki & Kirchner 1968 1969) This was verified in the present study

The internal branch of the SLN is the most important afferent pathway for reflex laryngeal closure. Reflex responses were elicited in the RLN at minimum threshold and latency (Fig. 3 B) Stimulation of the external branch of the SLN also evoked the reflex response in the RLN showing a higher threshold and a long period of latency and duration (Fig. 3 C)

Stimulation of the RLN itself elicited reflex responses in the same nerve (Fig. 3 D) It is, however very doubtful that these reflexes originating in the external branch of the SLN or the RLN itself participate in the reflex protective mechanism of the larynx, because the resholds of these reflexes were extremely igh, measuring 20 times that for the internal branch. Further the response itself was rather feeble compared with that elicited by stimulation of other laryngopharyngeal afferents. Moreover the response showed variation in latency and duration, relatively few discharges, and these of low voltage In addition, the reflex response occurred chiefly in the lateral cricoarytenoid rather than in the thyroarytenoid muscle Responses were extremely feeble in the PCA muscle, and this was not true for any other afferents. It is possible that responses to stimulation of the external branch of the SLN or the RLN itself might serve as part of the cough reflex.

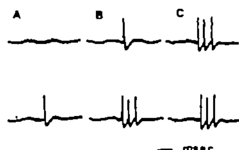


Fig 5 In small filament prepared from the nerve to the thyroarytenoid muscle reflex responses were elicited to stimulation of the internal branch of the SLN with gradually increasing voltage, demonstrating differences in number of nerve fibers which respond to stimulation with respiratory cycle. Upper tracings wer made in inspiration and lower in expiration. (A) With stimulation at very low voltage (0.05 V and 0.1 msec), only one fiber responds in expiration, but none in inspiration. (B) With a little higher voltage (0.07 V and 0.1 msec), the fiber responds in both phases and the other two fibers begin to respond only in expiration. (C) With supramaximal stimulus (0._ V and 0.1 msec), these three fibers responded in both phases. It is possible that the spikes represent repetitive discharges in the same fiber but this seems unlikely because of the short interval between them (? or 3 msec).

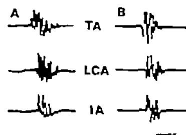


Fig 4 Reflex responses elicited in (A) the motor nerve and (B) the EMG of the thyroarytenoid muscle (*TA*), lateral cricoarytenoid (*LCA*) and interarytenoid (*IA*) to stimulation of the internal branch of the ipsilateral SLN (0.5 V 0.1 msec). EMG was recorded after sectioning RLN and SLN on the opposite side and after denervation of the other muscles on the same side to eliminate possible artifact due to current spread. Latency time is 7–8 msec, 12–14 msec and 12–13 msec respectively It should be noted that LCA and IA responded well to every single shock stimulus without facilitation by repetitive stimuli.

2. *Reflex responses of each laryngeal muscle to stimulation of the internal branch of the superior laryngeal nerve*

1 *Thyroarytenoid muscle* The thyroarytenoid muscle responded most intensely and consistently to stimulation of the internal branch of the SLN with a short latency and a long duration. The responses appeared both in the motor

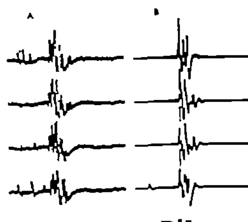


Fig 6. Reflex responses in (A) the motor nerve and (B) the EMG of the posterior cricoarytenoid muscle elicited by stimulation of the internal branch of the SLN (0.5 V and 0.1 msec). Recordings were made continuously from the left side by stimulating the ipsilateral SLN after sectioning the RLN and SLN on the opposite side and denervating the thyroarytenoid, interarytenoid and lateral cricoarytenoid muscles of the same side. Latent period is the longest (13–15 msec), and duration the shortest (10–12 msec) of all the laryngeal muscles. Spontaneous inspiratory activity can be seen in A, but not in B because of the low gain at which the recordings were made.

nerve twig and in the EMG (Fig. 4) During supramaximal stimuli the respiratory cycle did not influence the intensity of response. With submaximal stimuli, on the contrary the responses were more intense during expiration than inspiration indicating that more muscle fibers respond to stimulation during the expiratory phase (Figs. 5 10) The same phenomena were observed in a small filament to the thyroarytenoid muscle Fig. 5 shows that low voltage stimulation elicited the reflex in one fiber of this filament in expiration, but not in inspiration. With a little higher voltage the fiber responded in both expiratory and inspiratory phases and two other fibers began to respond during expiration. These three fibers responded to every stimulus during supramaximal stimulation without any relation to the respiratory cycle None of the fibers to the thyroarytenoid muscle was more likely to respond during inspiration. The results indicate that each nerve fiber to the thyroarytenoid muscle has a different threshold for the reflex and all of the fibers respond more easily during expiration than inspiration.

In this study we have never observed spontaneous activity related to quiet respiration, either in the thyroarytenoid muscle nor in its motor nerve, though several authors have reported such activity (Faaborg-Andersen, 1965 Green & Neil, 1955 Nakamura et al., 1958 Weddell et al., 1944 Yagi, 1963) We have observed thyroarytenoid muscle activity during expiration only when the level of anesthesia was so light that the animal phonated with each expiration.

2. *Lateral cricoarytenoid muscle* Reflex responses were elicited in the lateral cricoarytenoid muscle and also in its motor nerve twig with each stimulation of the internal branch of the SLN (Fig. 4) As in the case of the thyroarytenoid muscle the reflex was more easily driven during expiration, probably the result of facilitation (Fig. 10). The threshold for the reflex was about twice as high as for the thyroarytenoid muscle.

3 *Interarytenoid muscle* The interarytenoid muscle is poorly developed in the cat, being

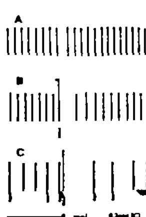


Fig 7 Reflex responses in very small filament prepared from the nerve to the posterior cricoarytenoid muscle (not from the RLN). (A) Pre-existent inspiratory activity in one fiber in this filament is reflexly inhibited by stimulation of the internal branch f the SLN at very low voltage (0.08 V and 0.1 msec). (B) With little higher voltage (0.2 V and 0.1 msec), reflex inhibition in the same filament continues longer and reflex discharge from another fiber with different voltage begins to take part. (C) The same responses are shown at higher speed recordings.

composed only of the transverse part situated above the PCA muscle Stimulation of the SLN however evoked responses both in the interarytenoid muscle and in its motor nerve even with single shock stimuli. Repetitive stimuli produced no facilitation. The threshold for the reflex was very low almost the same as for the thyroarytenoid muscle (Fig. 4) The reflex in this muscle showed no preference for expiration, the same voltage being required in both phases of respiration In properly prepared cases the reflex contraction of the muscle could be observed directly through the surgical microscope

4 *Posterior cricoarytenoid muscle* Spontaneous inspiratory activity was observed in the PCA muscle. Stimulation of the internal branch of the SLN evoked two kinds of reflex responses in the muscle. The first was a reflex inhibition of the inspiratory activity the second a reflex contraction of the muscle Both were clearly demonstrated in the EMG of the muscle and in its motor nerve after denervation of all the adductor muscles (Fig. 6) The latency of the reflex discharge was the longest and the duration the shortest among the laryngeal muscles. When the reflex was elicited during inspiration it was followed by cessation of

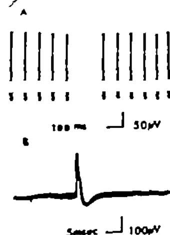


Fig 8. The nerve twig to the posterior cricoarytenoid muscle contains two kinds of fibers, each with different type of response to stimulation of internal branch of the SLN (A) The fiber which is active on inspiration is reflexly inhibited. (B) The other kind of fiber does not show respiratory activity but discharges reflexly



Fig 9 Reflex inhibition of inspiratory activity in a single nerve fiber prepared from the nerve twig to the posterior cricoarytenoid muscle, showing variation in its duration with strength of stimulus, applied to the internal branch of the SLN Same phase of inspiration in all three tracings. Stimulus strength as follows. (A) 0.1 V 0.1 msec produces inhibition of 150 msec. (B) 0.3 V 0.1 msec produces inhibition of 700 msec. (C) 0.5 V 0.1 msec produces inhibition of 300 msec. Similarly duration of inhibition varied with the phase of inspiration when strength of stimulus was unchanged. Inhibition was longest near the end of inspiration, shortest in mid phase

inspiratory discharges (Fig. 7) Latency for reflex inhibition was almost the same as for reflex activation or occasionally a little shorter

The nerve twig to the PCA muscle was divided into many small filaments, one of which, showing inspiratory activity was placed across the recording electrodes. Stimulation of the SLN with very low voltage elicited reflex inhibition of this inspiratory activity for a very short time. With a little higher voltage the reflex in the same fiber was inhibited for a longer time and a reflex discharge from another fiber in the filament began to appear (Fig. 7) Another filament with an inspiratory activity showed only an inhibition and no reflex discharge even on stimulation at a higher voltage. On the contrary a third filament showed no spontaneous activity but was reflexly activated (Fig. 8) These results indicate that the nerve to the PCA muscle is composed of two functionally different kinds of fibers, each responding differently to laryngeal stimulation. Those fibers showing inspiratory activity are easily inhibited at almost the same threshold required for reflex contraction of the thyroarytenoid muscle The other type does not discharge with respiration but it is activated reflexly at a

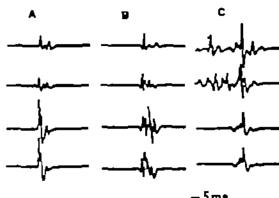


Fig. 10. Reflex responses in EMG of (A) thyroarytenoid, (B) lateral cricoarytenoid and (C) posterior cricoarytenoid muscles elicited by stimulation of the internal branch of the SLN with submaximal stimulus, demonstrating differences in degree of responses with respiratory cycle. Upper two tracings were made in inspiration and lower two in expiration. The thyroarytenoid muscle, stimulated at 0.07 V and 0.1 msec, showed higher degree of responses in expiration, which indicates that more fibers respond to stimulation in expiration than in inspiration. The lateral cricoarytenoid muscle stimulated with 0.15 V and 0.1 msec, also show greater response during expiration. The posterior cricoarytenoid muscle, stimulated with 0.2 V and 0.1 msec, shows higher responses in inspiration. Note spontaneous inspiratory activity in the posterior cricoarytenoid muscle in upper two tracings (C).

threshold about three times higher than that of the thyroarytenoid muscle.

Reflex inhibition of inspiratory activity varies according to stimulus strength and level of inspiration (Fig. 9).

Submaximal stimulation evoked a feeble reflex discharge in the EMG of the PCA muscle. In this situation the voltage of the discharge was always higher in inspiration than in expiration, indicating that more muscle fibers respond to stimulation in the inspiratory phase (Fig. 10 C). The same phenomena were demonstrated in a small filament prepared from the nerve to the PCA muscle. As shown in Fig. 11 afferent stimulation (SLN) at low voltage elicited a reflex discharge in one fiber of a filament to the PCA during inspiration but not during expiration. With a little higher voltage the fiber responded in both phases and another fiber began to respond during inspiration. With supramaximal stimulation both fibers responded well in both phases. The results indicate that each fiber has a different threshold for reflex activation and responds more easily during inspiration.

Excitability of the motoneuron pool for reflex activation of the PCA muscle appears to be mediated by an intricate mechanism in the brain stem as evidenced by a further observation. when the frequency of afferent stimulation (SLN) was increased gradually up to 10/sec, the adductor muscle responded to every stimulus. The reflex discharge in the PCA muscle, on the other hand, subsided gradually and became completely silent during stimulation at 10/sec or over. The same phenomena were observed in the nerve recordings which indicates that the inactivity of the PCA muscle to repetitive stimulation of the afferents is not due to exhaustion of the muscle or its neuro-muscular transmission but is due to an accumulated inhibitory process at a higher level. With

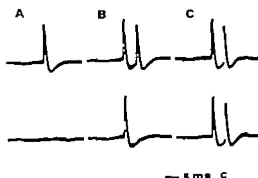


Fig. 11. Reflex responses were elicited in a small filament prepared from the nerve to the posterior cricoarytenoid muscle (not from the RLN) by stimulation of the internal branch of the SLN with gradually increasing voltage. These results indicate the influence of the respiratory cycle on the number of nerve fibers which respond to stimulation. Upper tracings were recorded in inspiration and lower in expiration. (A) Reflex response elicited in fiber by stimulation at very low voltage (0.1 V and 0.1 msec) only in inspiration. (B) With little higher voltage (0.. V and 0.1 msec), the fiber shows response in both phases and another fiber begins to respond in inspiration but not in expiration. (C) With supramaximal stimulus (0.5 V and 0.1 msec), both fibers respond in both phases. It seems unlikely that the discharges originate in one fiber because of the short interval, about 5 msec.

afferent stimuli over 10/sec the PCA muscle responded only to the first few stimuli and was inhibited thereafter

DISCUSSION

Stimulation of the internal branch of the SLN elicited a reflex response, either discharge or inhibition, in every laryngeal muscle including the abductor

Each fiber of the PCA muscle responded to stimulation more readily during inspiration, whereas fibers to the thyroarytenoid responded more readily during expiration. It seems possible that the respiratory center exerts a regulatory control over both sets of muscles, and that motor nucleus of the nerve fibers supplying the adductors is controlled by facilitatory and inhibitory influences from the medullary respiratory neurons, as with the intercostal motoneurones (Eccles et al 1962 Sears, 1964) Facilitation to the adductor motoneuron pool is apparently greater during expiration, being subliminal during quiet respiration. For this reason, the adductor responds to afferent stimuli more readily during expiration.

Similarly those abductor fibers which re to afferent stimulation by discharging apparently facilitated during inspiration, but insufficiently to discharge spontaneously

The challenging question, then, is what function, if any is served by reflex contraction of the PCA muscle in response to laryngeal stimulation. Could it augment glottic closure by adding tension to the adducted vocal cord, in cooperation with the adductor muscles? In any case, the functional relationship between the laryngeal muscles is not a simple antagonistic arrangement between the abductor and the adductors.

ZUSAMMENFASSUNG

Reflex Reaktionen wurden in dem rucklaufenden Kehlkopfnerv hervorgerufen durch elektrische Reizung an irgend einem Sinnesnervenstamm im Kehlkopf und Schlund Gebiet.

Elektrische Reizung am inneren Ast des überliegendem Kehlkopfnervens verursachte:

1 Reflex Reaktion in jeder Kehlkopfmuskel, die hintere Ringgiessbeckenmuskel einbegriffen.
2 Wegführung des Stimmbandes nach der Deuerung des Anführers.
3 Hemmung der Reaktion in Fasern des Motornervens zu der hinteren Ringgiessbeckenmuskel welche, während des Einatmens, entladete.
4 Beständige EMG Reaktionen in anführenden Muskeln nach Erregung des inneren Astes der überliegendem Kehlkopfnervens, mit Frequenzen über 10 per Sekunde. Die hintere Ringgiessbeckenmuskel reagierte nur nach den ersten Erregungen mit diesen Frequenzen, und wurde dann total gehemmt.
5 Stärkere Reaktion, bei dem Ausatmen den anführenden Muskeln, und bei dem Einatmen in der ausführenden Muskel.

Die Schwelle die latente Periode, und die Länge der Reflex Reaktion ist verschieden für jede Muskel und für jede Faser in ihren Motornerven.

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A SIMPLE METHOD TO MEASURE THE CILIARY BEAT RATE OF RESPIRATORY EPITHELIUM

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(Received September 14 1970)

Abstract It appears to be possible to correlate an acoustic rattle with a visual vibratory movement. This correlation has been used in order to measure the ciliary beat rate of respiratory mucous membrane. The accuracy of this subjective measurement is a function of the frequency and is proportionally constant up to 25 Hz. The standard deviation is about 10% Above 25 Hz the correlation becomes unreliable Interchange of octaves does not occur By means of this method the ciliary beat rate can be measured in the clinic in a simple way on large groups of patients.

From observation of the oscillograph, the otolaryngologist is accustomed to the fact that und is registered by the eye He may find e converse phenomenon surprisingly useful. the rapid movement of small objects is detected by the ear Use of this method of examination is therefore quite suitable in our specialty since our relation with audiology makes the equipment readily available This paper deals with an examination of ciliary action.

It is well-known that the tapis roulant of the mucus on the respiratory mucous membrane plays an important part in the defence mechanism against external, harmful influences. The function of the system is mainly deter mined by two factors. firstly by the activity of ciliated cells secondly by the character and the composition of the mucus layer Therefore an examination of the function of the tapis roulant" and of the factors that affect it, should be directed both towards the activity of the ciliated cells, and towards the nature and the composition of the mucus. Our interest was especially aroused by the viscosity of the mucus. We hope to discuss this on a later occasion.

Ciliary activity is often judged in practice by the transport speed of the tapis roulant But under pathological conditions one cannot decide whether this is the result of a disordered ciliary activity a change in the character of the mucus, or an abnormal anatomical variation. Therefore this method provides insufficient information for clinical use. For direct observation in vivo only a small part of the nasal mucosa is available

Consequently one is almost dependent on the method of examination in vitro Experience has shown, however that the behaviour of ciliated epithelium in vitro—provided that the environment conforms to certain conditions—bears a marked resemblance to the behaviour in vivo (Lucas, 1933)

For the examination of the mucous membrane the clinician would be greatly served by a method that would lend itself to routine measurement by virtue of its simplicity In our laboratory such investigations have been in progress for some time.

METHOD

A small part of the epithelial lining of the mucous membrane to be examined is scraped

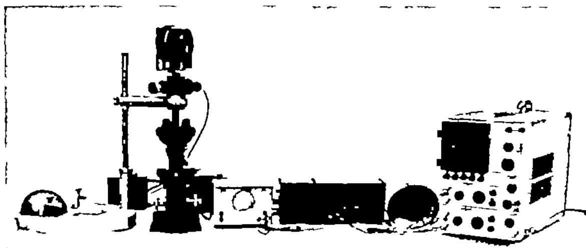


Fig 1 Set-up for the estimation of the correlation. *Left:* phase-contrast microscope with filming apparatus. *Centre:* sawtooth-generator amplifier loudspeaker *Right.* oscilloscope (on the screen static sawtooth).

off with the aid of a small sharp curette, in the nose from the posterior part of the inferior turbinal, from the trachea or the bronchi during bronchoscopy This scraping off is traumatic to such a limited extent that—as far as the nose is concerned—it can be carried out without using any anesthetic.

A part of the curetted epithelial fragment is immediately immersed in Gey solution, pH 7.2, which is contained in a ring (diameter 1 cm, thickness 0 25 mm) that is placed upon an object-glass. The liquid-chamber in which the fragment of tissue is embedded, is then closed with a cover-glass and inspected under the phase-contrast microscope at room-temperature (22 C) Especially along the folds of the epithelium, but also when viewed from above, ciliary movement is quite apparent under the mucus layer which is usually still present. The remaining fragment of tissue is fixed and used for histological and histochemical examination.

The difficulty is now how to measure the ciliary beat rate in a simple way and how to record it in a reasonably reliable and comparable numerical value. A number of relevant methods are to be found in the literature on this subject. The most reliable method is to make a record on a high-speed film and to calculate the beat rate on the basis of the known filming speed (Dalhamn, 1960 Ewert, 1965) This method, however is too elaborate and too expensive for routine use. The method of Ballenger & Orr (1963) based on the measuring of the ciliary activity by the use of the rotation velocity of spherical fragments obtained from a tissue culture, is laborious and, in our

Table I *Measuring results of the estimation of the correlation*

Frequency in Hz	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Number of measurements	7	6	8	16	17	12	18	8	15	7	6	4	8	8	6
S D Δf	0 8	1.3	1 5	1.3	1 4	1 4	1 6	1 4	1 9	1 6	2.4	2.3	2.3	2 9	1.8
Relative S.D	8	12	13	10	10	9	10	8	10	8	12	11	10	13	8

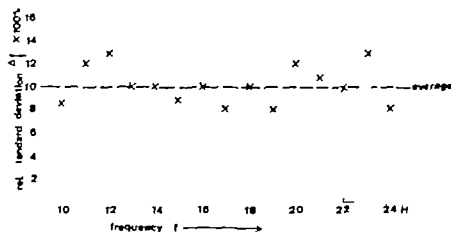


Fig 2 Relative standard deviation as a function of frequency

opinion, not suitable. On their way the rotating fragments frequently meet obstacles which disturb the rotation.

In our experience stroboscopy is unreliable at the low frequency of 6–20 beats/second. Moreover due to metachronicity and phase difference between the different groups of ciliated cells, it is very difficult to make an accurate estimation.

We have tried to solve the problem of measuring the ciliary beat rate by comparing the beat rate as observed visually with a rattle observed auditorily which is conveyed to a loudspeaker with the aid of a sawtooth-generator By choosing the frequency of the rattle in such a way that it corresponds as closely as possible with the rhythm observed visually the frequency of this corresponding rhythm can be read off from the frequency dial of the generator

In order to test this method as to its reliability we have imitated the ciliary movement, as it is to be seen through the phase-contrast microscope with the moving dot of an oscilloscope By bringing this dot out of focus—by maladjusting it—a band is to be seen moving along the screen with a rhythm to be chosen voluntarily With the rattle described above one can next try to choose auditorily a frequency in such a way that it corresponds as closely as possible with the band rhythm as observed visually on the screen of the oscilloscope. The chosen frequency can be read off from the generator-dial. By connecting the generator with the vertical plates of the oscilloscope the accuracy of the chosen frequency can be checked (Fig. 1) Correctly a static picture can be seen in case of a faulty adjustment the exact frequency is to be found by turning the frequency-dial until a static picture returns. The difference in frequency of the two dial positions is a measure for the inaccuracy of the subjectively adjusted value.

RESULTS

Table I and Fig 2 give an overall picture of the results obtained in this way on the basis of almost 150 measurements carried out by different examiners The very high correlation-coefficient and the relatively slight standard deviation justify the utility of this extremely simple measurement of the ciliary beat rate which is manageable in the clinic as a rapid routine method.

In a series of 30 patients with allergic rhinitis and positive provocation test the average beat frequency was 13 beats sec. Just before

and immediately after the provocation test we could find no difference in beat frequencies.

Fifteen Patients with a chronic rhinitis showed a much slower beat frequency of an average of 7 beats/sec.

However when the preparations were washed with Gey solution in such a way that much of the mucus was removed from the surface the beat frequency in both series rose *to an equal level of 15 beats/sec.*

In a later publication we hope to return to the clinical application of this method.

ZUSAMMENFASSUNG

Es stellt sich als möglich heraus, ein akustisches Rasseln mit einer visuellen Schwingungsbewegung zu korrelieren. Von dieser Korrelation wurde Gebrauch gemacht, um die Flimmerschlagfrequenz respiratorischen Epitheliums zu messen. Die Genauigkeit dieser subjektiven Messung ist eine Funktion der Frequenz und ist bis zu 25 Hz prozentual konstant. Die Normaldeviation ist ungefähr 10%. Über 25 Hz wird die Korrelation unzuverlässig. Oktavenverwechslungen kommen nicht vor. Mit dieser Methode kann in der Klinik auf eine einfache Weise die Flimmerschlagfrequenz bei größeren Gruppen von Patienten gemessen werden.

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CLONAL PATTERN OF METASTASIS IN A CASE OF MALIGNANT MUCOEPIDERMOID TUMOUR OF THE PALATAL SALIVARY GLAND

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Abstract A case of metastasizing mucoepidermoid tumour of the palatal salivary glands is presented. The disease was characterized by a protracted course of local recurrences, metastases in regional lymph nodes and an uncontrollable distal spread at the terminal stage Some of the metastases were histologically pure mucus-producing adenocarcinomas and some were pure epidermoid forms without glandular structures. Characteristic intermingled patterns were also seen. This clonal pattern is taken to indicate mosaic structure in the original tumour with subpopulations endowed with varying differentiative capacities. The existence of such almost monotypic forms of mucoepidermoid tumour is important to keep in mind in the practical work.

Mucoepidermoid tumours of the salivary glands form a group in which the diagnostic criteria are relatively unambiguous but in which it is difficult to evaluate the clinically malignant potential of the tumour Opinions concerning their malignancy vary in the literature. After earlier descriptions of mucoepidermoid tumours under varying titles (Schilling 19 1 Masson & Berger 1924 Skorpil 1940) Stewart et al. (1945) presented the first large collective series of 45 tumours classified separately as highly differentiated benign tumours and poorly differentiated malignant forms They pointed out the difficulty of evaluating borderline cases and suggested a third intermediate group to contain these. Eneroth (1964) collected a material of 23 mucoepidermoid tumours and concluded that the whole group should be considered as carcinomas, since no reliable criteria could be found to distinguish clearly the potentially malignant tumours from the benign forms, though the prognosis was much better in the well-differentiated group An opposing view has been taken by Kleinsasser (1969) who considers mucoepidermoid tumours benign and only in exceptional cases capable of malignant behaviour

Histogenetically these tumours are considered to originate in the intra and interlobular duct epithelium with differentiative capacities to mucus-secreting papillary structures on the one hand and to epidermoid differentiation on the other Intermingled between these cell types are found characteristic intermediate cells and other less common cell forms. Without taking issue with the questions of the classification quoted above, we present a case of mucoepidermoid tumour of the small salivary glands of the hard palate which demonstrated these various differentiative pathways in its pattern of metastasis over a prolonged period of time.

Clinical history

The original tumour reddish-blue in colour had been removed from the left side of the hard palate in 1945 when the patient was 19 years old. Radiotherapy had been given postoperatively the dose being 2 048 rads. The surgeon examined the biopsy specimen himself and was of the opinion that an adamantinoma was in question. In 1966 a reddish-blue tumour was

Table I *Predominant pattern of histological types in the metastases of a palatal mucoepidermoid tumour*

Designation	Time of excision	Location	Salient histological features
A	January 11 1966	Left subauricular node	Rich mucus-producing tissue and intermediate cells. N epidermoid components
B	June 29 1967	Local recurrent tumour	Epidermoid, well differentiated tissue with few intermediate cells but no mucus production
C	August 18 1967	Left subauricular tumour	Epidermoid, well differentiated tissue and few intermediate cells. Growth mainly intra-lymphangiatic
D	September 1967	Supraclavicular node	Epidermoid, well differentiated tissue. No mucus production
E	September 1967	Retro-orbital node, left	Epidermoid and islands of richly mucus producing tissue and intermediate cells
F	November 22, 1967	Preauricular node, left	Mainly mucus producing partly cystic tissue and some islands with epidermoid differentiation
G	March 12, 1968	Local recurrent tumour	Rich mucus production, many intermediate cells and intermingled keratinisation of individual cells and small islands
H	July 8, 1969	Subcutaneous metastases	Altogether 7 metastatic nodules, and all of these have well differentiated epidermoid morphology with few intermediate cells and no mucus production
I	July 15 1969	Subcutaneous metastatic tumours with various sites on the trunk and left upper arm	

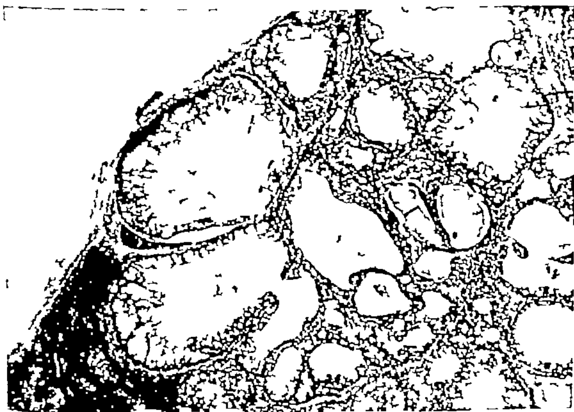


Fig 1 Lymph-node metastasis with pure mucus-producing pattern with some intermediate cell forms. Van Gieson, 16.



Fig. 2. A soft-tissue metastasis demonstrating a practically pure epidermoid type. Van Gieson, ×700.

ain seen in the same place as before on the left side of the hard palate. The patient himself reported that this tumour had the same appearance as the one removed in 1945. At the same time a hard gland was noticed in the neck, behind the left mandibular angle. The gland was excised and diagnosed as metastasis of mucoepidermoid carcinoma. The palatal tumour which histologically proved to be a mucoepidermoid carcinoma was treated with Roentgen therapy. Soon after this therapy in 1966 however it was found that the tumour had spread over an unexpectedly wide area. For this reason, resection of the left and right maxilla, and a radical neck dissection on the left side were carried out. In 1967 a recurrence was noted in the cavity left by the earlier operation, and was treated with electrocauterization. Subsequently the growth of the tumour could no longer be controlled despite extensive extirpations, radiotherapy and chemotherapy. In the final phases of the disease there were widespread metastases, and the patient succumbed in 1969. No autopsy was carried out.

Histological features

The original tumour biopsy specimen of 1945 has not been traced despite great efforts. All the available histological material is compatible with the diagnosis of mucoepidermoid tumour of the salivary gland. The salient features of each individual metastasis and recurrence are recorded in Table I in order to focus the attention on the variability of the morphology. Tumour metastases designated as A, F and G (Table I) all from different locations consisted mainly of the mucoid component of the tumour and the first of these consisted entirely of this



Fig. 3. Detail of a metastasis exhibiting mainly the epidermoid pattern but clear mucus-producing cells are intermingled as well as characteristic intermediate cells. Van Gieson, 16.

tissue (Fig. 1) On the other hand metastases designated as B C, D and all the islands of the final distant spread, were composed entirely of epidermoid islands of metastatic tissue with no mucus production and only few islands of inter mediate cells (Fig. 2) Intermediate forms of these two monotypic" forms (Fig. 3) were also found as demonstrated in metastases de signated as E, F and G

Although the growth of the tumour in all of these instances was clearly infiltrative, the grade of differentiation of the tissue was always high, the islands were solid and well demarcated while mitoses and nuclear atypism were rare. The latter features were more pronounced in the latest metastases representing distant spread of the tumour to various locations

Concluding remarks

The case described proves an unquestionably malignant course of the disease for mucoepider moid tumour of the salivary gland The inter pretation of the whole history of the patient is invalidated to a certain extent by the una ab ability of the original tumour which had been removed from the hard palate 21 years prior to the start of the present history and class as an adamantinoma. This classification in fact have been wrong since at the criteria for classifying mucoepidermoid were not quite settled. In the materi salivary gland tumours from the of Otolaryngology Helsinki Univer Hospital, 2 of 6 patients with fied as mucoepidermoid were

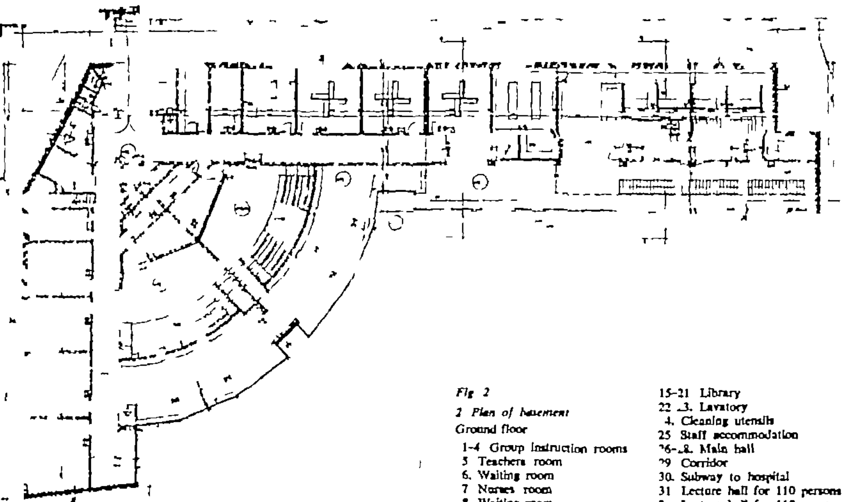


Fig 2

2 Plan of basement

Ground floor

1–4 Group instruction rooms
5 Teachers room
6. Waiting room
7 Nurses room
8. Waiting room
9 Nurses room, utoclave
10 Teachers' room
11 14 Group instruction rooms
15–21 Library
22 .3. Lavatory
4. Cleaning utensils
25 Staff accommodation
76–.8. Main hall
79 Corridor
30. Subway to hospital
31 Lecture hall for 110 persons
3.. Lecture hall for 110 persons
33 Washroom, autoclave
34–35 Teaching materials
36. Washroom

Fig 3 Otolaryngology lecture hall with televisions



Fig 4 Otolaryngology group-instruction ... off by cross-partitions. Four patients are ... d ... ined by the medical students and ... round giving instruction

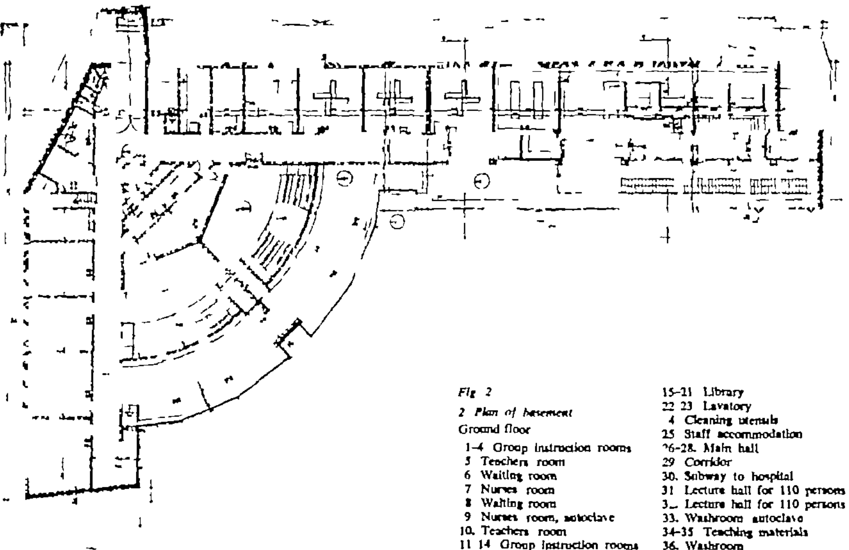


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USE OF TRIANGULAR WAVEFORMS OF ANGULAR VELOCITY IN THE STUDY OF VESTIBULAR FUNCTION

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bstract. According to theoretical models of the vestibular response system, measures of few response *arameters can be used to predict the dynamic* haracteristics of vestibular responses of individuals variety of situations. Therefore, these measures ave theoretical significance as important clinical ols and also as means of predicting individual reac ons in aerospace operations. The present paper deals ith methods for assessing several of these parameters at relate t semicircular canal function, and in articular it elucidates the development of method at appears to be practical for the reliable measurement of both nystagmus and sensation parameters. he results of several experiments involved in the evelopment of the method are described.

Aviators frequently encounter unnatural ac elerations that elicit misleading vestibular sensations. These sensations can produce disorientation, i.e inaccurate perception of the atti ude or state of motion of the aircraft, which he pilot must overcome or suppress by the use f other available sources of information. Typically pilots are successful in this endeavor However individuals differ in sensitivity to estibular stimuli and in their ability to suppress vestibular sensations. Vestibular disorders, especially recent or paroxysmal disorders, can introduce extreme individual differences that can alter ability to cope with the unnatural stimuli of flight. Since these kinds of differences among pilots can be significant factors in air crew safety and performance, their assessment in pilot candidates and pilots troubled by ver tigo are important tasks for aviation medicine.

Measurement of nystagmus is generally ac cepted as significant in the clinical evaluation of vestibular function, but measures of vestibular sensations may also be significant in the examination of flight personnel because aviators must contend with these sensations in flight. Moreover directional asymmetry in vestibular turning sensations was reported to be present more frequently in pilots whose pre senting symptom was vertigo than in other flight personnel (Benson, 1967) Directional asymmetry in nystagmus does not necessarily indicate directional asymmetry in sensation (Benson, 1967) and reliable departures be tween measures of sensation and nystagmus have been demonstrated for certain stimuli (Guedry 1965 pp 68–80) Thus it appears that neither nystagmus nor sensation can be used as a valid indicant of the other and that reliable measurement of both may reveal aspects of function which would not be indicated by either measure alone

Many recent technological advances enhanced the feasibility of objective

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ment of nystagmus for clinical purposes, but the reliable measurement of vestibular sensations by methods that are practical for large scale clinical assessment of pilots and pilot candidates is still a challenging problem. Methods of measuring vestibular sensation known as "magnitude estimation" (Brown, 1966; Clark & Stewart, 1968) provide estimates of change in sensation magnitude and hence they are potentially useful for measuring system parameters such as "time constants of response decay" (Π/Δ) and "adaptation time constants" (τ). However, these methods clearly are not valid measures of differences among individuals in absolute sensation magnitude and hence they cannot measure a parameter which might be called "system gain" K (θ/Δ). Several methods of estimating subjective angular velocity involving continuous judgments of either displacement or velocity (von Békésy, 1955; Cawthorne et al., 1956; Groen & Jongkees, 1948; Guedry, 1965) theoretically could provide estimates of these three system parameters, but most subjects require practice to yield what appear to be meaningful results. When administered in a manner that would be practical for large-scale clinical assessment, these methods seem to be unreliable. Conventional sensation cupulometry introduced by van Egmond (van Egmond, 1949; van Egmond et al., 1948; van Egmond et al., 1949) and in current use by Groen (1969), also has practical limitations. This procedure is useful for the assessment of the Π/Δ time constant in clinical cases by an experienced examiner. However, when used in a more routine manner practical for broader testing, both its reliability (Guedry & Owens, 1967) and its validity in predicting flight performance (Dobie, 1969) have been questioned. For these reasons, alternative methods for assessing vestibular sensations are being explored.

Several recent experiments indicate that reliable measures of vestibular sensation can be obtained by the use of triangular waveforms of angular velocity and a particular method of obtaining subjective reports (Guedry et al., 1970; Owens & Guedry, 1969). The present paper describes the theoretical basis for the use of this stimulus in the evaluation of semicircular canal function and also describes some results obtained by this method.

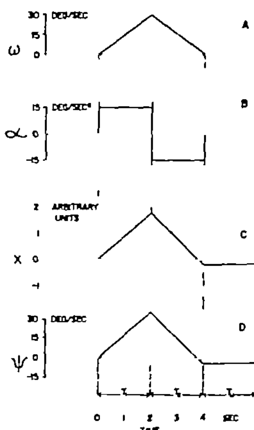


Fig. 1. Predicted responses during a short triangular waveform stimulus. Cupula displacement (x) and subjective angular velocity (ψ) in panels C and D respectively resemble stimulus angular velocity (ω) waveform in panel A and not angular acceleration (α) waveform in panel B. Values of $\Pi/\Delta = 16$ sec, and K $(\theta/\Delta) = 19.9$ sec were used in Eq. (1) to generate the ψ curve.

Theoretical Basis for Using the Triangular Waveforms as a Semicircular Canal Stimulus

It is widely believed that cupula displacement regulates the rate of discharge of the ampullary nerves, utriculopetal displacement augmenting and utriculofugal displacement diminishing the firing rate relative to a spontaneous resting level of activity in the ampullary nerves of the horizontal canals (Benson, 1965). In turn, the neural firing rate is believed to regulate the

magnitude of responses such as nystagmus and subjective angular velocity subject to further control by central neural mechanisms It is therefore reasonable to use theoretical cupula displacement during semicircular canal stimulation as an approximate predictor of nystagmus and turning sensations.

The particular stimulus of interest here is a triangular waveform of angular velocity which consists of angular acceleration in one direction, a_1 followed immediately by angular acceleration of equal magnitude in the other direction, a_2, as shown in Fig. 1 If only the case in which the acceleration (a_1) and deceleration (a_2) are of equal magnitude is considered, then an approximate predictive equation for responses such as nystagmus slow phase velocity (ψ_s) or subjective angular velocity (ψ_s) derived from the torsion pendulum theory (van Egmond et al 1949 Groen, 1956) is.

$$\psi = \propto \left(K\frac{\theta}{\Delta}\right)$$

$$\left[\left(2-\exp\left(-\frac{\Delta}{\pi}t\right)\right)\exp\left(-\frac{\Delta}{\pi}t_s\right)-1\right]\exp\left(-\frac{\Delta}{\pi}t_w\right) \tag{1}$$

where K is the constant of proportionality between the particular response (either ψ or ψ) and cupula deflection, where θ Δ, and Π are, respectively the inertial, the spring action, and the viscous damping constants of the cupula-endolymph system, and where t t_s, and t are measures of time within T_1 T and T_w, as defined in Fig. 1

Triangular waveforms of brief duration approximate the type of stimulus received by the canals during natural movement. Under these circumstances the form of the theoretical cupula displacement curve is believed to be similar to the form of the stimulus angular velocity curve as shown in Fig. 1 This illustrates the rationale for the statement (Benson, 1965 Jones et al., 1964) that, although the cupula-endolymph system responds to angular acceleration, it performs as an angular velocity transducer for stimuli approximating natural head movements.

If the semicircular canals provide angular velocity information like that shown in Fig. 1 and if subjects are able to integrate this information over time accurately then the integral of the area under the ψ curve should predict subjective displacement estimates (ψ_s) By integration of Eq (1) from the beginning of T_1 until the time of the response zero crossing in T (see Fig. 1) a predictive equation for subjective angular displacement ψ can be obtained.

$$\psi = \infty\left(K_s\frac{\theta}{\Delta}\right)$$

$$\left[T_1-\frac{\pi}{\Delta}\ln\left(2-\exp\left(-\frac{\Delta}{\pi}T\right)\right)\right] \tag{2}$$

This equation simplifies to the following form

$$\psi_s = \infty\left(K_s\frac{\theta}{\Delta}\right)(T_1-t) \tag{3}$$

where t_r is the time in T_2 of the response 'zero-crossing' and it was derived by setting $\psi = 0$ in Eq (1) and solving for t_s. Thus,

$$t = \frac{\pi}{\Delta}\ln\left(2-\exp\left(-\frac{\Delta}{\pi}T_1\right)\right) \tag{4}$$

Although we are primarily concerned with the measurement of sensation in the present paper the nystagmus response provides a convenient illustration (Fig. 2) of the particular response characteristics for which the above equations were developed. ψ ψ and t each has nystagmus and sensation analogs. The slow phase velocity (ψ_s) curve (Fig. 2 *C*) is probably similar in form to its subjective angular velocity (ψ_s) analog, but as indicated earlier there are practical limitations on the reliable measurement of ψ_r. The other two response characteristics, t and ψ seem to offer opportunities for reliable measurement in both the nystagmus and sensation. The reversal of nystagmus during T_2, apparent in Fig. 2 B and C, provides a fairly definite measure of t_r. Its subjective counterpart can be obtained from signaled

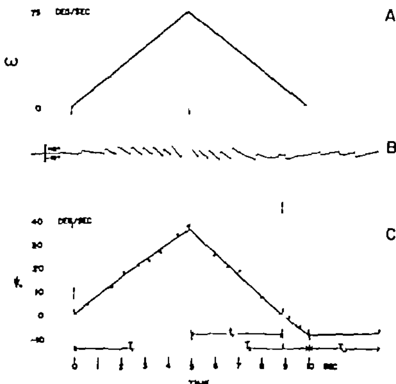


Fig. 2. Typical nystagmus response (panel B) during a triangular waveform of angular velocity (panel A). Nystagmus slow phase velocity ($\dot{\psi}$) and the measurement of t are shown in Panel C.

points of reversal of apparent rotation and such measures correspond fairly closely to nystagmus measures when stimulus waveforms are short (Guedry 1965). Summation of nystagmus slow phase displacement during the interval $T + t$ which can be accomplished electronically (Guedry & Turnipseed 1968) provides a measure of ψ. Its subjective analog, ψ_s, can be obtained from estimates of angular displacement. In the following section the development of a method of measuring ψ is described.

Development of Method for Reporting Subjective Angular Displacement

When rotation about an Earth-vertical axis is passive and accomplished under conditions to minimize visual and other sensory data about an external reference, the semicircular canals are probably the primary source for judgments of angular displacement. Triangular waveforms of angular velocity producing relatively short arcs of rotation were selected as stimuli because they approximate natural stimuli. It was hypothesized that judgments of such stimuli would be reliable and consistent, since the subjects would be operating within a range of naturally occurring stimuli.

The stimulus series comprised ten triangular waveforms of angular velocity of different wavelengths to produce angular displacements of 15, 60, 135, 240 and 375 in both the clockwise and counterclockwise directions. a and a_s were ± 15 deg/sec² for all stimulus waveforms.

The rotation device was a Stille Werner RS-3 rotator leveled so that the axis of rotation was vertical, and modified by attaching a concentric cylindrical light proof enclosure (6 feet in diameter) to the rotary structure. The subject's head was positioned by occipital rests at the center of rotation so that the lateral semicircular canals were approximately in the plane of rotation. Wide band noise was applied through audiometric headsets to mask auditory localization cues.

Initially two methods of obtaining subjective data were employed.

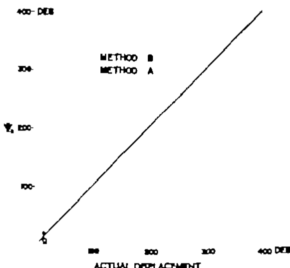


Fig 3 Mean subjective displacement estimates (ψ_E) obtained with two methods of reporting.

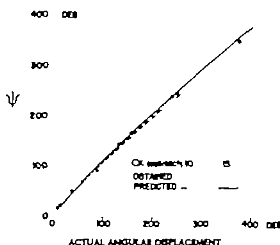


Fig 4 Mean retrospective estimates of angular displacement obtained with the dial shown in relation to theoretical curves for -10 deg/sec² and -15 deg/sec² when $K(\theta/\Delta) = 19.9$ sec and $\Pi/\Delta = 16$ sec.

Method A

A spot of light was projected approximately at eye level onto a white strip that conformed to the inner wall of the cylindrical enclosure The subject was instructed to turn the light source in a compensatory direction so that the spot would remain at a fixed position in space In the event that the perceived turn exceeded the excursion limit of the light source (120 degrees), subjects were to reposition the light and recommence tracking.

Method B

The subject viewed a circular dial (10 inches in diameter) marked off in 10-degree intervals. The enclosure was dimly illuminated by a small light over the subject's head. The face of the dial was in the Earth-horizontal plane and was supported just above the subject's lap so that it was viewed with a downward-directed gaze and at a reading distance of about 14 inches. The subject was instructed to move a pointer on the dial in a compensatory direction so that the pointer would maintain a fixed compass heading.

Eleven subjects participated in the experiment. Six of these were administered the stimulus series first using Method A and again using Method B The order of the methods was reversed for the other five subjects.

Estimates made using Method A were significantly smaller than estimates made using Method B. This is apparent in Fig. 3 Introspective comments of the subjects suggest a reason for this difference. When they used Method A, subjects reported that counterrotation of the light spot (which appeared at eye level) seemed to diminish the subjective impression of body rotation. *It is to be noted* that the directions of the light and eye velocities relative to the skull are the same, and if these velocities are matched in magnitude, then the image of the light spot will be fixed on the retina. Thus, the nystagmus slow phase would not be impeded by the visual stimulus, nor would there be appropriate slow movement of the image over the retina. This kind of visual–vestibular–proprioceptive information, where the visual target produced neither suppression of vestibular nystagmus nor retinal smear while it was being moved voluntarily may have caused the reduced estimates of body displacement indicated with Method A but this is conjectural and is discussed in more detail elsewhere (Guedry et al., 1969)

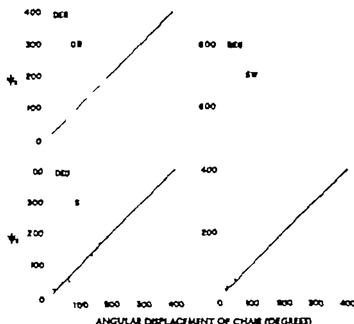


Fig 5 Individual differences in relationships between estimates of angular displacement (ψ) and actual angular displacement.

An important feature of the results is that both methods yielded a progressive underestimation of angular displacement as actual displacement increased. At first this result might be considered a sign of adaptation (assuming an average Π/Δ of about 16 sec), but observation of the way in which subjects performed eir tasks suggests a different interpretation. bjects had been instructed to move the light spot (Method A) or the pointer (Method B) in a compensatory direction to maintain it in a fixed compass heading. It was apparent that *many subjects moved the pointer simultaneously* with the changing subjective velocity but others delayed until the stimulus was completed or was almost completed before making an estimate. In the latter case subjects were making retrospective displacement judgments, whereas in the former subjects were attempting to make velocity-matching judgments at least part of the time Concurrent velocity matching involves a different process than does retrospective displacement matching, and furthermore, the concurrent psychomotor performance required in velocity matching could interfere with the processing of incoming sensory data.

In a second experiment, this ambiguity in the subjects mode of operation was avoided by instructing them to wait until the initial effect was completed before giving their displacement estimates. In addition, because there is some sensation of reversed motion following the stimulus (as would be expected from cupula reversal during T_2 shown in Fig. 1), subjects were specifically instructed to make their judgments quickly just after the point of reversal and to ignore the sensation of reversed motion.

Method A (movement of the light spot) was *discarded and all subjects reported their responses* using Method B (movement of the pointer on the circular dial) in the second experiment.

As before, the stimuli were triangular wave forms of angular velocity with the increase and decrease in speed in any one waveform accomplished by angular acceleration of the same magnitude and duration. Angular accelerations of two magnitudes, 10 and 15 deg/sec² were used to produce the velocity changes, and as a result, a given stimulus duration gave two different angular displacements.

Twenty-six subjects participated in the second experiment. Each subject was administered

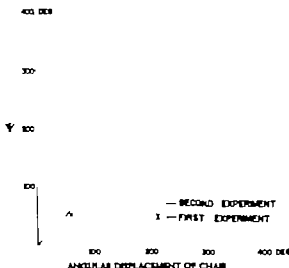


Fig 6 Mean estimates of identical stimuli obtained from different groups of subjects in the two experiments. Instructions led to concurrent velocity matching in the first experiment and required retrospective displacement estimates in the second.

a twenty-trial series of stimuli comprising ten clockwise and ten counterclockwise trials. The order of presentation of stimuli was scrambled within the series, but it was the same for each subject. Subjects made their estimates quickly and only 15 to 20 sec intervened between stimuli, thus a series of twenty stimuli was presented in about 7 min. After completing the series, the subjects were given a rest and then the series was presented again in order to gain an estimate of test–retest reliability

The results, shown in Figs. 4 and 5 illustrate that *the mean estimates of angular displacement* were nearly accurate. Separate theoretical curves were plotted for the 10 and 15 deg/sec² stimuli to illustrate the fact that only slight differences in ψ were expected for these two stimulus magnitudes within the range of stimulus wavelengths used. These curves were calculated from Eq (2) assuming K $(\theta/\Delta) = 19.9$ sec and $\Pi/\Delta = 16$ sec.

Results from three subjects are shown in Fig. 5 It is apparent that each of these individuals gave consistent responses, but differed markedly from one another in the relationship of their responses to actual angular displacement. These three individuals are fairly representative of the range of responses of the entire sample. Individual consistency within series is indicated by high correlations between actual and perceived displacement. Mean correlation was 0.95 indicating an orderly increase in displacement estimates as actual displacement was increased for most individuals tested.

To estimate test–retest reliability slopes of the lines of best fit through the raw data points of each subject were determined for the first series of stimuli and the second. The correlation between slopes from the first and second series was 0.93 for clockwise trials and 0.87 for counterclockwise trials. When the estimates from clockwise and counterclockwise trials were combined, the correlation between slopes obtained from the 26 subjects for the two series was 0.94 Thus it is clear that, within one experimental session at least, when a subject yields a given slope in one series, he is apt to yield a similar slope in a second series.

The close correspondence between actual and mean estimated displacement found in the second experiment is to be compared with the results from the first experiment when Method B (movement of the pointer on a dial) was also used. As illustrated in Fig. 6, the two sets of data diverge as the angular displacement increases. The mean estimates of the 375-degree displacement were 206 degrees and 351 degrees in the first and second experiments, respectively and this difference is statistically *significant* ($t = 3.45$ $df = 35$ $p < .01$). It appears likely that the improved accuracy in the second experiment is attributable to the requirement placed upon subjects to make retrospective judgments, i.e., to delay judgment until the apparent initial displacement was complete. This difference has important implications for the development of vestibular adaptation models (Malcolm, 1970 Young & Oman, 1969) because a response decline that might otherwise be attributed to a strong vestibular adaptation effect appears to be the result of the reporting procedure chosen.

DISCUSSION

The consistency of retrospective displacement judgments indicates that these men were able to integrate immediate subjective angular velocity ($\dot{\psi}_s$) over time of the triangular waveforms used here to arrive at systematic estimates of angular displacement (ψ_s) The accuracy of the mean ψ estimates suggests the mean subjective velocity profiles during these stimuli were also fairly accurate. Based on theory presented in an earlier section, Eq (2) should provide an adequate description of the ψ responses in fact, it does, as was shown in Fig. 4 Assuming that the theory is adequate these responses can be used with others to determine parameters of the vestibular response systems.

Estimation of K (θ/Δ)

The constant, $K_s (\theta/\Delta)$, can be determined for individual subjects from measures of ψ during a sequence of triangular waveforms. Aside from specifiable stimulus variables, there are three unknowns in Eq (3) ψ_m $K (\theta/\Delta)$ and t_r. Both ψ and t are measurable subjective responses and therefore, when both of these are measured, $K (\theta/\Delta)$ can be determined. The same procedure can be used in estimating $K(\theta/\Delta)$, when total nystagmus slow phase displacement, ψ_m, and t have been measured (as illustrated in Fig. 2) Alternatively if an estimate of Π/Δ for an individual is already available from some other procedure, t can be calculated from Eq (4) and then $K (\theta/\Delta)$ can be estimated without the direct measurement of t_r.

Estimation of Π/Δ

Response to triangular waveforms can also be used to estimate Π/Δ for individuals. Note that in Eq (4), t is determined by Π/Δ and measurable aspects of the stimulus. Therefore, substitution of t into Eq (4) provides a means of estimating Π/Δ for individuals. This can be done with t measures based on either subjective signals of turning points or nystagmus turning points (Guedry 1965 p 70) The simplicity of the measurement of t from nystagmus records is illustrated in Fig. 2 above. It is probably preferable to use nystagmus for this determination because it would be less affected by adaptation effects (Young & Oman, 1969)

Advantages of estimating Π/Δ by this method, as opposed to several cupulometry procedures, are that the endpoint determinations seem to be easy reliable, and not time consuming. However some of these advantages are offset somewhat by the theoretical significance of errors associated with the measurement of t_r. As illustrated in Fig. 7 slight difference in t during a short triangular waveform (e.g., $T_1 = T_2 = 5$ sec) implies a substantial difference in Π/Δ therefore, errors of measurement are critical. With longer triangular waveforms, the measurement of t is accomplished as easily as with short waveforms moreover the resolution of Π/Δ by differences in t is much more favorable Therefore long triangular waveforms should be used to measure t_r. Unfortunately longer waveform stimuli increase the possibility that estimates of Π/Δ will be distorted by adaptation effects (Guedry 1970; Young & Oman, 1969) Recent articles (Malcolm, 1970; Young & Oman, 1969) have sought to provide mathematical descriptions of these adaptation effects, and these models predict that estimation of Π/Δ through t measurements during long triangular waveforms would be distorted by adaptation. However Π/Δ obtained by conventional cupulometry would be altered on the same basis and, as a matter of fact, Groen (1956) considers this to be the likely explanation of the differences in Π/Δ as estimated from nystagmus and sensation cupulograms.

Estimation of adaptation time constant (τ)

The clear presence of adaptation effects in responses of long duration (Guedry 1965 pp. 72–80) and their potential influence on estimates of response parameters emphasize the importance of attempting to describe mathematically the nature of the adaptation process. An accurate mathematical description of the ef

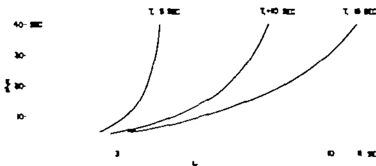


Fig 7 Illustrating the improved resolution of Π/Δ from t measures as T is increased.

fects would permit mathematical corrections of estimates of Π/Δ for some test procedures or alternatively it would permit specification of test procedures in which adaptation is avoided. Mathematical deductions from a proposed adaptation model (Young & Oman, 1969) predict that t measures for sensation and nystagmus would first increase and then decrease as stimulus wavelength is increased. Thus the shape of the function $t = f(T)$ may serve as a measure of individual differences in adaptation time constants for sensation (τ_s) and for nystagmus (τ) If and should prove to have real meaning as parameters of individual response systems, then in addition to expanding the universe of accurate theoretical prediction, these measures would have clinical significance by providing indications of neurophysiological processes not related to the other parameters.

SUMMARY AND CONCLUSIONS

One of the primary purposes of this paper was to evaluate triangular waveforms of angular velocity and the responses they elicit as a means of assessing individual differences in vestibular sensations. Triangular waveforms were selected as test stimuli because they approximate stimuli that are frequently experienced in natural movement, permitting subjects to operate within a range of stimulation and judgment for which they are naturally practiced. Thus it was hypothesized that these judgments would be reliable and that difficulties encountered in the past with unnatural stimuli might be avoided. The consistency of individual judgments obtained in the experiment supports this hypothesis.

The methods described here provide theoretically valid estimates of several vestibular system parameters. If the theory is adequate, these parameters can be used to predict vestibular sensations for a wide range of stimulus conditions and they should be useful in clinical diagnosis. However it remains to be shown that subjective responses to natural stimuli have diagnostic significance. Natural stimuli afford natural practice and from this practice, compensatory mechanisms may reduce diagnostic sensitivity to old lesions If so, then other stimuli such as long, unnatural triangular waveforms which still offer advantages of measurement, may prove more fruitful. For this reason, it is desirable to test the usefulness of the measures used here in detecting vestibular disorders with clinical material, including flight personnel disturbed by aviator's vertigo.

ZUSAMMENFASSUNG

Gemäss dem theoretischen Modell des vestibulären Reaktionssystems können Messungen einiger Reaktionsparameter dazu benutzt werden, die dynamischen Charakteristiken vestibulärer Reaktionen von Individuen unter verschiedenen Bedingungen vorherzusagen. Diesen Messungen kommt deshalb theoretische Bedeutung zu, weil sie als wichtige klinische Anzeichen und auch als Hilfsmittel der Voraussage individueller Reaktionen in Flug- und Raumuntersuchungen angewandt werden können. Die vorliegende Arbeit untersucht Methoden, einige dieser Parameter die sich auf die Bogengangsfunktion beziehen, abzuschätzen. Sie beschreibt insbesondere die Entwicklung einer Methode die für die zuverlässige Messung

nystagmischen wie auch von Wahrnehmungs-Parametern nützlich erscheint. Die Ergebnisse mehrerer Experimente bezüglich der Entwicklung der Methode sind beschrieben.

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GALVANIC NYSTAGMOGRAPHY

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Abstract. During binauricular stimulation with the cathode on the right and the left tragus, using d.c. constant current increasing from 0 to 1 600 μA, the velocity of the slow component of the elicited nystagmus was traced using Torok's photocell connected direct to a potentiometer writer The speed of the slow component was found to be a linear function of the current strength. As a measure for the difference between the sensitivity on the right and the left side galvanic index (GI) is outlined. The GI-value expresses the quotient between an increase of φ°/sec/mA on the two sides. The distribution of the GI-values in normal subjects is found to be Gaussian. It is also found that the difference between the within-subjects variance and the between-subjects variance is not significant.

In 1909 Mackenzie applied galvanic stimulation to the human vestibular system. He observed the elicited eye movement directly In the same year Buys described a pneumokymographic method by which the movement configuration was recorded at a relatively lower current stimulation. Pfaltz introduced the galvanic test in clinical routine work, employing his photonystagmographic technique in 1957 The aim of the present study was to adapt and extend this technique for clinical purposes.

MATERIAL AND METHODS

Subjects

In this work we used normal subjects in the age range 15 to 50 years. In order to ensure that the subjects had vestibular normality it was required that they had no history of vestibular diseases and that normal responses were found to rotation test, and to the Hallpike caloric test. In addition, further otoneurologic investigations including a test for positional nystagmus observed behind Frenzel's goggles did not reveal any abnormalities.

Stimulation

The constant current generator used, supplied by an 18 V battery permits stimulation from 0–3 000 μA in 100 or 200 μA steps in an area with variable electrode impedance from 0 to about 6 kΩ.

The electrodes consisted of flat silver discs, about 3 cm² wrapped in chamois which had been immersed in tap water By means of a helmet, the cathode and anode may be fitted in bushings on holders which are pressed against the skull (Fig. 1)

There is a free choice of electrode combinations. For binauricular stimulation B 1 cathode on the left side, anode on the right B 2. vice versa (Fig. 2)

The tragus was selected as the best site for the electrodes, since empirically a stronger nystagmoid reaction is elicited by this means than by using the mastoid process.

Recording

In front of one eye, Torok's (Nyklel & Torok, 1963) photocell aggregate was placed, mounted on the same helmet as the stimulation elec

This work was supported by the Swedish Medical Research Council. (B-68-21X 173-02.)

Fig 1 Helmet with lectrodes and photocells mounted on patient.

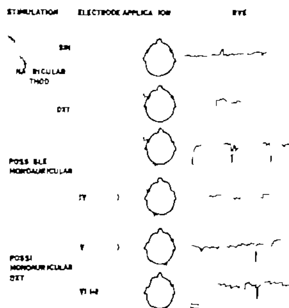


Fig 2 Nystagmus direction t various electrode applications. In this investigation the electrode-position was binauricular There are four other possibilities in the so-called monoauricular which was not used here.

trodes (Fig. 1) The output from the photocells is so high (25–50 mV per 1 bulb deviation) that recording on a Servogor DC potentiometer writer is possible without pre-amplification. This DC writer has a balanced input impedance of 1 MΩ and a frequency range from DC to 10 Hz. The combination of photocells and writer has such a high sensitivity that a speed of eye deviation of 0.1 /sec can be recorded. Using a calibration of ±10 and a suitably short photocell distance from the eyeball, so that blinking does not interfere, it is easy to make 1 correspond to 5 mm in the 50 mV area on the writer

Fixation

Spontaneous eye movements and deviation in darkness sometimes interfered with the galvanic current-induced nystagmus. We therefore found it useful to apply when needed for short moments, a dim blue spot of light which made the eyes return to mid-position. After removal of

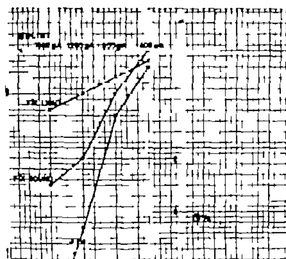


Fig. 3 Influence of fixation with light and sound

this light, the galvanic nystagmus could be reproduced. Fixation of light suppresses spontaneous as well as induced eye movements (according to Pfaltz 5 to 10 times) (Fig. 3). The following calculations were based on those segments of the curves which represent stimulation in complete darkness.

Procedure

The helmet with the electrodes and photocells was placed on the patient's head. The patient sat down in a chair in complete darkness. The further procedure was as follows. (1) Calibration of patient with equipment ±10. (2) Stimulation B 1 from 400 μA to 1 600 μA. (3) Repeated calibration. (4) Repeated stimulation, this time with B 2.

The entire investigation takes 5 minutes.

The parameter

Apart from the direction of the nystagmus there is a distinct alteration in frequency, amplitude and speed of the slow eye deviation as a function of increase in stimulus intensity (Figs. 3, 4).

The response on the right and the left side in binauricular stimulation is assessed by evaluating the ratio between the increase in degrees per sec per 1 mA on the two sides. We decided to call this the galvanic index (GI).

The GI value can then be calculated as the ratio between the slopes of the two lines corresponding to either direction of the current. The ratio is expressed as a decimal number larger than or equal to 1.0 with an index (sin or dxt) denoting the direction associated with the larger slope.

Fig. 5 shows 3 normal curves for 3 different subjects. The slopes are variable but uniform on the two sides.

Statistics

This work includes three parts of statistics.

(1) To state the relation between the stimulus and the reaction, our observations corresponding to measurements in a given direction on a given subject were attempted, described by a simple linear regression model with current strength as the independent and the speed of the slow component as the dependent variable.

(2) We tried to fit the normal values by a

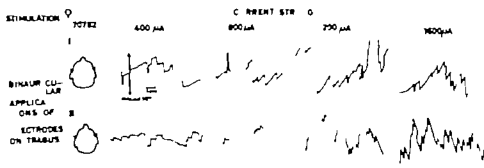


Fig. 4 Reaction with increased current.

Fig 1 Helmet with electrodes and photocells mounted on patient.

Fig 2 Nystagmus direction at various electrode applications. In this investigation the electrode-position was binauricular There are four other possibilities in the so-called monoauricular which was not used here.

trodes (Fig. 1) The output from the photocells is so high (25–50 mV per 1 bulb deviation) that recording on a Servogor DC potentiometer writer is possible without pre amplification. This DC writer has a balanced input impedance of 1 MΩ and a frequency range from DC to 10 Hz. The combination of photocells and writer has such a high sensitivity that a speed of eye deviation of 0 1 /sec can be recorded. Using a calibration of ± 10 and a suitably short photocell distance from the eye ball, so that blinking does not interfere, it is easy to make 1 correspond to 5 mm in the 50 mV area on the writer

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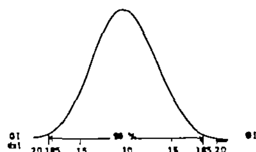


Fig 7 Theoretical Gaussian distribution for normals.

F-test we demonstrated that the difference between the two variances was *not* significant.

The two variances were pooled to form an estimate of the within-subjects variance of the GI values. This was compared with the between-subjects variance by a *F*-test and found not to differ significantly

DISCUSSION

Throughout the past 170 years galvanic methods have been in the limelight for clinical use (Purkinje, 1820 Hitzig, 1871 Mackenzie, 1909 Buys, 1909 Brunner 1943 Bellens, 1950 Pfaltz, 1968 Breson & Krag, 1967), but such use has never been found dependable. After the turn of the century few authors (Mackenzie, 1909 Gabersek & Jobert, 1965 Pfaltz, 1968) have dared draw firm conclusions from their testing on human subjects. The reasons why this method has been in disrepute in relation to the caloric as well as rotatory tests have been pointed out by Frenzel (1957) and Jongkees (1953) The mode of stimulation was inaccurate, and in principle the recording was inapplicable when using ENG owing to superposed voltage variations as demonstrated by Gabersek & Jobert (1965) Pfaltz (1968) and Breson & Krag (1967). In the present study we were able to stimulate with constant current through the integuments and could eliminate varying electrode–skin resistance. At the same time, recording by photocells in front of one eye could hardly be rendered more sensitive in a conventional set-up" for clinical use.

Table I. *The distribution of 45 observations*

Between GI and GI 5 minutes
Between GI and GI 1 month

	GI		GI_1		GI	
15 persons	DXT	SIN	DXT	SIN	DXT	SIN
♀ J N 10 08 53	1.21			1.23	1.02	
♂ O R. 07 09 51	1 13			1.37		1.04
♀ L. G. 03 08 33		1 15	1.36		1.12	
♀ R. E. 29 08 50		1 41		1.01		1 16
♀ J K. 23 01 39	1.50		1.18		1 73	
♀ B. L. 23 03 53	1.16		1.00			1.51
♀ B. C. 18 03 53	1.21		1.04			1.19
♀ B. R. 15 05 47	1.14			1.05	1.16	
♀ B. L. 16 06 50		1.54		1.52		1 16
♂ R. E. 01 03 53		1.52	1.12		1.31	
♀ G R. 06 02 50		1.02		1.36		1.03
♀ M. R. 01 02 50	1.39		1.12		1.14	
♂ K. J 20 10 40		1 43		1.01		1.12
♂ J S. 10 09 46		1.02		1.64	1.27	
♀ S. N 30 03 51		1.36		1 12		1.05

Mackenzie (1909) stimulated by silver-ball electrodes on the temporal bone. The balls, wrapped in chamois, were moistened with physiological saline and were alternately anodes and cathodes. The other electrode was placed in the patient's hand. The patient sat in the dark and fixed a point several meters behind the investigator who observed one of the patient's eyes which was illuminated by a reflector A nystagmus reaction was observed in normals from 1.5 mA to 7 mA The direction of the nystagmus was as previously reported by Hitzig (1871) who was the first to give an objective description of eye movements under the influence of galvanic current. In addition to studying increasing and decreasing strength

of current, Mackenzie used sudden connections and interruptions of the current. The most important observation in normals was the relatively uniform reaction on both sides, there being at most" a difference of one mA.

In labyrinthectomized subjects stimulated by current of a strength right up to 16 mA (during which some of the patients became somewhat uneasy"), a reduced reaction was found on the affected side. However this side did react to so-called anode stimulation.

Our mode of stimulation differs from Mackenzie s, and a direct comparison of the results is not permissible. The recording of the nystagmus also differed.

However we were able to state that normal subjects show approximately the same slope of the curves on both sides. The standard deviation of the values in normal subjects is low

Brunner in 1943 felt that he could distinguish between a centrally and peripherally conditioned spontaneous nystagmus, when he exposed the patients to galvanic vestibular stimulation. Peripherally conditioned nystagmus could be reduced when the positive pole was placed in the direction of the nystagmus. These findings cannot yet be confirmed by our studies.

Bellens in 1950 performed galvanic stimulation on 50 normal subjects. He observed nys us behind Frenzel s goggles and reported t only 22% showed a difference of less than 5 mA between the two sides at the "threshold value By monoauricular stimulation it was not possible to elicit any reaction at all in more than 12% not even at 5 mA. He concluded that the galvanic vestibular test was hardly of any stable clinical value· first, the electrode–skin resistance alternated secondly the current density in the vestibular system was not known thirdly it was not known in which part of the system the current acted and fourthly the reaction also depended upon the duration of stimulation.

Pfaltz (1968) operates with the concept 'latency period With our stimulation we laid no stress on this, but only upon gradually increasing intensity in the reaction in normals as well as in abnormal cases with lacking or reduced ability to respond. Using rather large electrodes, pressed manually against the skull, Pfaltz could apply gradually increasing strength of current up to 8 mA, although this did cause the patient some discomfort. By a possibly pure DC recording Pfaltz used in normals the parameters amplitude (0.3–1 9 /beat) and frequency (0 2–1 8 beats/sec) to indicate the nystagmus reaction. We found similar values, but we used only the change in the speed of eye movement as a measure In addition, Pfaltz used the so-called reversal phenomenon in sudden reverse poling and thereby reversal of the nystagmus reaction into the direction opposed to the previous one. We omitted this because of the influence of the reverse poling phenomena upon the course of the reaction and thereby upon the GI ratio

Conclusion

The most important reasons why the galvanic method has not yet been used in neuro-otological diagnostics are (1) a non-dependable mode of stimulation, and (2) of recording, as well as the fact that (3) it is not yet known, as mentioned by all students of this subject, at which site in the vestibular system the DC acts, despite extensive animal experiments (Dohlman, 1929 Ledoux, 1949 Löwenstein, 1955) We have not set up any working hypothesis concerning the target of the current, but have proceeded in a traditional clinical manner and have investigated, by so-called black box tests, whether it is a all possible to induce an applicable reaction by galvanic stimulation. In our opinion it is. Unlike a number of previous authors, we are not prepared to draw any conclusion which may prove conjunctive The final proof as to where the target of the current is will hardly be advanced in a clinical study

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I want to thank the Direction of Gentofte County Hospital for favourable working conditions and N -G Henriksson, the vestibular laboratory E.N T Department, University of Lund, Sweden, for most valuable help.

ZUSAMMENFASSUNG

Während einer binauralen Reizung mit der Kathode am rechten und linken Tragus unter Verwendung von konstantem Gleichstrom, der von 0 bis auf 1 600 μA erhöht wurde, wurde die Geschwindigkeit der langsamen Komponente des ausgelösten Nystagmus mit Hilfe von Toroks Photozelle, direkt verbunden mit einem Potentiometerschreiber aufgezeichnet.

Es wurde festgestellt, dass die Geschwindigkeit der langsamen Komponente eine lineare Funktion der Stromstärke ist.

Als Masstab für den Unterschied zwischen der Empfindlichkeit auf der rechten und linken Seite wird der galvanische Index (GI) dargestellt. Der GI Wert drückt den Quotienten zwischen einer Erhöhung um φ°/sec/mA auf beiden Seiten aus.

Es wurde festgestellt, dass die Verteilung der GI-Werte bei normalen Personen den Gauss'schen Zahlen entspricht.

Es wurde ferner festgestellt, dass die Schwankungen zwischen verschiedenen Tests bei ein und derselben Person von der gleichen Grössenordnung sind, wie die Schwankungen zwischen verschiedenen Personen.

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VESTIBULAR NERVE RESPONSE TO PRESSURE CHANGES IN THE EXTERNAL AUDITORY MEATUS OF THE GUINEA PIG

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(Received October 14 1970)

Abstract Increases and decreases of action potential (pulse) rat were observed in some single vestibular ganglion nerve cells of the guinea pig in response to pressure changes in the external auditory meatus. Minimum stimulus intensities necessary to elicit clear responses were in the range of 1 2 cm Hg. These results (1) support an hypothesis regarding mechanisms of vestibular activation by intense infra- and audiofrequency sound, and (2) suggest a new stimulus technique for neurophysiological investigations of the vestibular pathway and central sensory integration.

A considerable body of evidence indicates that the vestibular apparatus can be activated by gh intensity low frequency acoustical stimulaon (Camis, 1930 Parker et al. 1968) Recently a possible mechanism was postulated to account for acoustical vestibular stimulation whereby an average displacement of the stapes footplate produces excitation of the vestibular receptors (Reschke et al. 1970) In the case of high intensity audiofrequency sound stimulation, this shift in the average position of the stapes footplate is assumed to be caused by asymmetrical motion of the ossicular chain as has been observed in human cadavers and live cats (Guinan & Peak, 1967). In the case of static pressure or infrasound pressure changes in the external auditory meatus, the motion of the stapes is assumed to occur as a direct response to the pressure stimulation rather than as a secondary effect of nonlinear distortion.

Experimentation undertaken in guinea pigs to examine this hypothesis indicated that pressure stimulation at intensities of 2.5–10 cm Hg and frequencies of 0 1–10 Hz could elicit three general types of eye movements. oscillatory eye movements, counterrolling,[1] and nystagmus (Parker et al., 1968) In subsequent experimentation, head movements associated with pressure stimulation were found to be significantly correlated with eye movements (Reschke et al., 1970) The results of these investigations are interpreted as supporting the hypothesis that stapes displacement, and consequently perilymph/endolymph displacement, produces direct mechanical stimulation of the semicircular canal receptors.

The present study was undertaken because it was believed that the interpretation of results from the previous experiments would be strengthened by direct neurophysiological evidence. Therefore an experiment was designed to see if responses from primary vestibular afferent nerve cells could be related to air pressure changes in the external auditory meatus.

METHOD

Useful data were obtained from 13 guinea pigs which were anesthetized with sodium pentobarbital. The animals were restrained in a head

[1] Counterrolling, in this report, refers to an eye movement which is analogous (that seen when a guinea pig is rotated around its cephalocaudal axis.

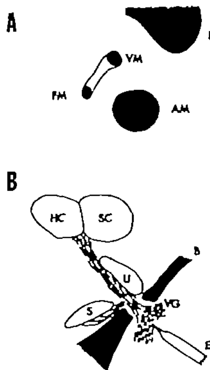


Fig 1 (*A*) illustrates medial wall of guinea pig right temporal bone. Depressions or holes, indicated in black, include vestibular nerve meatus (*VM*), facial nerve meatus (*FM*), auditory nerve meatus (*AM*) and cavity for paraflocculus (*F*). (*B*) schematically illustrates that the vestibular ganglion (*VG*) contains nerve fibers which innervate the horizontal canal (*HC*), superior canal (*SC*), utricle (*U*) and saccule (*S*). *B* indicates bone and *E* indicates the electrode.

holder by ear bars and a tooth clamp. The stimulus was introduced through the right ear bar which was hollow The stimulus source was a 5 cc hypodermic syringe and was connected to the ear bar with plastic tubing. Stimulus amplitude and onset rate were determined by the magnitude and rate at which the experimenter moved the syringe plunger A Statham pressure transducer (Model P 23 A) provided a continuous record of the pressure changes and a manometer was used for stimulus calibration.

Fig. 1 *A* illustrates a portion of the medial wall of the guinea pig right temporal bone. Part of the vestibular nerve enters the cranial cavity through the vestibular meatus (VM) Fig. 1 *B* illustrates that the portion of the vestibular nerve which enters through the vestibular meatus contains cells that innervate the saccular and utricular maculae and the horizontal and superior semicircular canals. The cell bodies of the vestibular ganglion (Scarpa s) are located at the mouth of the vestibular meatus. The location of the electrode (*E*) in the vestibular ganglion is also illustrated.

The tip of the recording electrode (3 M KCl 2 μ tip diameter) was located at the mouth of the vestibular meatus by means of a set of stereotaxic coordinates based on the distance between the vestibular meatus and the ear bar tip. The electrode was inserted through an opening in the dorsal-lateral quadrant of the skull on the side contralateral to the recording site. The electrode track was in the same frontal plane as the vestibular meatus and at an angle of 40 elevation from the horizontal plane. Because of anatomical variability successful electrode placement was achieved with only about 50% of the subjects.

When single nerve cell responses were successfully isolated, changes in neural pulse rate as a function of stimulus intensity and duration were examined. Stimulus intensities ranged from 1 to 9 cm Hg of increased or decreased pressure. Durations varied from 1 to 20 sec. At least 1 min was allowed for recovery between stimulus presentations.

Signals from the pressure transducer and the electrode were amplified and recorded on magnetic tape. Subsequently the recorded signals were displayed on a dual beam oscilloscope and photographed with a Grass oscilloscope camera (Model C4)

All of the data presented in this report were derived from neurons which met the following criteria (1) located in or at the mouth of the vestibular meatus, (2) response amplitude at least twice as large as noise (3) not sound responsive as determined by rubbing or tapping the plastic tubing which connected the hypodermic syringe to the ear bar (which produced sound intensities at the tympanic membrane of up to 103 dB SPL) (4) regular
as determined with audio

change pulse rate with movement of electrode tip $\pm 10\ \mu$ as determined with audio monitor and (6) stable response for at least 15 min. The location of the electrode tip at the time of recording was determined by removing the electrode, removing the brain, and replacing the electrode at the same coordinates as those used when recording.

RESULTS

Observations have been made on 32 neurons that met the criteria noted above. Of these 32 neurons 11 gave a clear response to pressure stimulation, and the remaining 21 neurons could be activated by neither sound nor pressure. The responses to pressure consisted of clear increases or decreases of pulse rate. In some instances the changes in pulse rate were transient and in other cases the changes in pulse rate could be observed for durations up to 15 sec. Latencies of pulse rate change observed to date range from 0 1 to 5 sec.

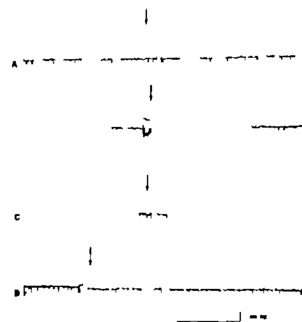


Fig 2 Example of neural response evoked by pressure changes in the external auditory meatus. For each record the upper trace indicates the pressure transducer output and the lower trace is the neural activity. The arrows indicate stimulus onset and termination. Stimulus parameters for record *A–B* 1.5 cm Hg intensity 4.6 sec duration. Stimulus parameters for record *C D* −1 5 cm Hg intensity 3.5 sec duration.

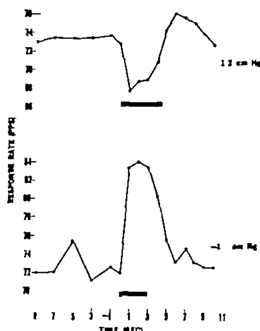


Fig 3 Average changes in neural response rate (pulses per second) following four presentations of pressure increases (upper curve) and two presentations of pressure decreases (lower curve). The abscissa indicates time in seconds before (negative values) and after stimulus presentation. The temporal location of the stimuli is indicated by the bars below the curves.

Fig. 2 illustrates the responses from a nerve cell which demonstrated a decrease in pulse rate following stimulation with increased pressure and increased pulse rate following pressure decreases. *A* and *B* represent responses evoked by a pressure increase of 1.5 cm Hg for approximately 4 6 sec. *C* and *D* illustrate responses evoked from the same neuron to a stimulus of −1.5 cm Hg intensity and 3.5 sec duration. In each record, the upper trace indicates the output of the pressure transducer and the lower trace is the amplified signal from the electrode. The arrows indicate stimulus onset and termination.

Changes in response rate following pressure increases and decreases are plotted in Fig. 3 The abscissa indicates time before (negative values) and after stimulus onset. The ordinate indicates the number of action potential responses that occurred within particular one

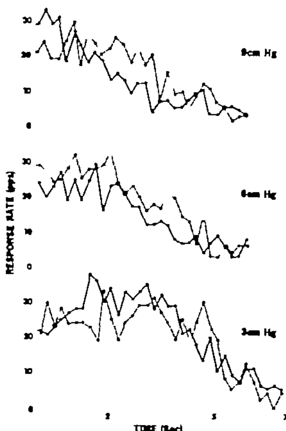


Fig. 4 Changes in response latency as a function of peak stimulus intensity *Abscissa* indicates time from stimulus onset in seconds. *Ordinate* indicates the number of nerve spikes which occurred within 0.2 sec intervals. The parameter in the figure is peak stimulus intensity The points connected by the dashed line indicate the response to the first presentation of the stimulus and the solid line indicates the response to the second stimulus presentation.

second time intervals (pulses per second) The black bars under each curve indicate the temporal position of the stimulus. The upper curve represents the average response to four stimulus presentations that ranged in intensity from 1 to 2 cm Hg and varied in duration between 4 and 5.5 sec The lower curve illustrates the average response to two stimulus presentations of approximately −1.5 cm Hg intensity and 3.5 sec duration. Stimulus rise and decay times were less than 0.5 sec The data in Fig. 3 were derived from the same neuron that is illustrated in Fig. 2.

Eight of the eleven drivable neurons observed in this study exhibited decreases in pulse rate to pressure increases of 1–9 cm Hg and little or no response to pressure decreases in the same range. The three remaining neurons exhibited increases and decreases in response rate as illustrated in Figs. 2 and 3

The data illustrated in Fig. 2 indicate short response latencies. Observations of other neurons indicate response latencies of up to 5 sec and that these latencies may change as a function of stimulus intensity An example of a long response latency neuron is presented in Fig. 4

DISCUSSION

The observations from the present experiment substantiate the view that pressure changes in the guinea pig's external auditory meatus can excite the receptors of the vestibular apparatus. Moreover these data support the hypothesis that a shift in the average position of the stapes footplate caused by such pressure changes or by intense acoustical stimulation can result in vestibular activation. Clearly however further work is required to compare the responses to intense sound and static pressure

The changes in neural pulse rate seen in this study correlate with the head and eye movement observations from our previous studies. Neural responses were evoked by the same range of stimulus intensities as the head and eye movements, and the time course of the *neural responses was congruent with the time* course of the head and eye movement responses observed previously in some instances.

Perhaps the most curious aspect of our research to date concerns the magnitude of response latencies that have been observed. Head and eye movement latencies of several seconds have been recorded in previous studies. Two neurons in the present study exhibited latencies of the order of 3–5 sec following low intensity stimulation. Possible mechanisms to account for these observations have been presented elsewhere (Parker et al., 1968 Reschke et 1970) Briefly we hypothesize the

changes in the external auditory meatus ultimately elicit fluid (perilymph and endolymph) flow through two pathways in the labyrinth. One pathway involves fluid displacement around the helicotrema or through the cochlear scala media resulting in deformation of the round window membrane. A second pathway allows fluid to be displaced through the endolymphatic duct resulting in deformation of the endolymphatic sac. We speculate that the second pathway has a much higher mechanical impedance than the first pathway and a correspondingly longer time constant. Therefore, the long latencies that have been observed may result from the mechanical characteristics of the hypothesized second fluid pathway The foregoing interpretation has two difficulties. First, how low velocity fluid displacement through the endolymphatic duct could produce activation of vestibular receptors is unclear Second, most of the neurons observed in the present study exhibited response latencies of considerably less than one second which would suggest a relatively low impedance fluid displacement pathway Experimentation is currently being pursued to clarify the problems raised by these investigations.

Several hypotheses have been considered to for the large proportion of nerve cells could not be activated by sound or pressure. We suggest that the non-drivable cells innervate the saccular and utricular maculae and that the cells which exhibit responses to pressure innervate the semicircular canals. This interpretation is supported by a previous study which indicates that head and eye movement responses to pressure are only slightly altered following centrifugal removal of the otoconia from the maculae (Reschke et al. 1970)

The stimulus techniques employed in this study should be of value for neurophysiological investigations of information transfer in the vestibular neural pathway and central sensory integration. Most previous investigators of vestibular responses have employed acceleration or electrical excitation as the stimulus (Camis, 1930· Brodal et al. 1962). When acceleration is used, it is difficult to maintain an electrode in a precise location because of the difference in specific gravity between the electrode and the surrounding tissue. The use of electrical stimulation has drawbacks because the current tends to spread away from the intended locus *unless rather exacting techniques* are employed, and it is difficult to know precisely which structures are activated.

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ZUSAMMENFASSUNG

Eine grosse Reihe Untersuchungen habt klar gezeigt, dass eine funktionelle Erregung des Vestibular-apparates durch nichtadequate Druckreize ausgelöst werden kann. Dieses Verhalten wurde sowohl für statische Änderungen, Druckänderungen im Infraschallbereich sowie auch für Hörschall mit hohen Intensitaten bei Tierversuchen und im Menschen nachgewiesen. In einer anderen Arbeit wurde ein Mechanismus postuliert, nach dem alle diese Erregungen des Vestibularapparates durch Druckänderungen derselben Ursache, nämlich einer Verschiebung der Ruhelage des Steigbügels, zu Grunde liegen soll. Um diese Hypothese zu prufen, wurden in der vorliegenden Untersuchung bei Meerschweinchen statische Druckänderungen im äusseren Ohrkanal erregt und die Antworten einzelner Ganglienzellen im N vestibularis registriert. Bei ungefähr einem Drittel der untersuchten Nervenzellen sind eindeutige Antworten auf die Erregungsreize beobachtet worden. Die Ergebnisse stützen also die Ausgangshypothese und eroffnen zusätzlich die Möglichkeit einer neuartigen Reizungstechnik für neurophysiologische Untersuchungen der vestibulären Nervenbahnen und der zentralen Sinnesintegration.

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THE CALORIC RESPONSE IN MENIERE'S DISEASE DURING SPONTANEOUS AND GLYCERIN INDUCED CHANGES OF THE HEARING LOSS

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Abstract In patients with Menière's disease the caloric response from the diseased labyrinth may vary during the course of the disease. Fluctuations in caloric response and fluctuations in hearing may occur independently After the consumption of glycerin by patients with Menière disease, the caloric response from the diseased ear may be reduced, reinforced or unaffected. Variations in caloric response and hearing have been compared; no distinct individual pattern has been noted. Possible reasons for the lack of agreement in the reaction pattern in the acoustic and non-acoustic labyrinth, both during the course of the disease and after glycerin consumption, are discussed. After glycerin consumption the caloric response was reduced on one side in one out of seven normal jects, while the hearing remained unaffected. Glylp may provoke direction-changing positional nysgmus in normal subjects as well as in patients with lenière's disease.

n cases of Menière's disease the administration of a single dose of glycerin (or glycerol) perorally may result in an immediate improvement in hearing (Klockhoff & Lindblom, 1966, 1967) The improvement which is observed primarily by pure-tone audiometry may be very marked in advanced cases in which the discrimination for speech is impaired and may lead to a considerable betterment in this discrimination, with the simultaneous elimination or reduction of phenomena such as distortion, tinnitus and stuffiness" (Klockhoff & Lindblom, 1967) However a reversion to the original condition is usually noted the following day

Since glycerin makes the blood hypertonic, this effect may be due to a transient, osmotically induced reduction of a labyrinthine hydrops. An improvement in hearing after taking a dose of glycerin may accordingly indicate the reduced influence of hydrodynamic damping.

In view of this, a glycerin test has been suggested, in order to obtain or to further confirm, by the possible finding of a reversible hearing loss, the diagnosis of Menière's disease and thereby to justify treatment with diuretics (Klockhoff & Lindblom, 1968) Successful results of the administration of peroral diuretics have been reported (Stahle, 1958 Norell & Stahle 1961 1962 Klockhoff & Lindblom, 1961 1967 1968) The rehabilitating effect was particularly manifest with respect to the vertigo, which is usually felt by the patient to be the most disabling symptom.

Starting from the momentary improvement of hearing which follows the consumption of glycerin and which is observed in about every other case of Menière's disease, we asked whether the vestibular function—expressed in terms of the caloric response—was also changed. Several observations have indicated

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that direct parallelism in cochlear and vestibular dysfunction cannot be expected. Thus, for example, attacks of dizziness may precede hearing loss for a long time and vice versa (Enander & Stahle, 1967 Klockhoff & Lindblom, 1967) and the caloric reaction may be normal, in spite of a considerable hearing loss (Stahle & Bergman, 1967 Enander & Stahle, 1969). These circumstances may mean that the vestibular dysfunction is not directly influenced by intralabyrinthine hydrops in the same way as the cochlear dysfunction, as can be shown by a glycerin test. In that case the symptoms of Menière's disease may be due to a disturbance that is still unknown but can partly be corrected by diuretics. Hydrops would then be *one consequence of this disturbance, of im*portance mainly as regards the hearing loss.

With this line of thought in the background, we shall present here the results of an investigation in which the non-acoustic and acoustic functions were compared, as they were reflected in the caloric response and the hearing. Comparisons were made (1) during a long period in the spontaneously fluctuating course of the disease and (2) in connection with momentary improvements in hearing induced *by glycerin consumption.*

MATERIAL AND METHODS

Our study comprises 55 patients with a characteristic history of the disease and typical audiometry together with a control group of 7 normal subjects. In 23 of the patients, the caloric response and the hearing loss were repeatedly studied over a long period. In the remaining 32 cases, comparisons between the vestibular and cochlear functions were made in connection with glycerin tests. These cases were, almost without exception, advanced, unilateral cases with reduced ability to discriminate for speech. Only 2 patients with bilateral symptoms are included in this group and in these 2 cases the study relates to the worse ear The 7 normal subjects were studied in connection with glycerin administration

Hearing test and glycerin test

In the long-term study the hearing loss was measured by ordinary pure-tone audiometry Glycerin tests were performed in the way previously described (Klockhoff & Lindblom, 1966, 1967) involving a single peroral dose of glycerin (1.2 cc/kg body weight) well chilled and mixed with an equal quantity of physiological saline solution, together with drops of lemon juice to correct the taste.

Immediately before the glycerin was consumed, Békésy audiograms (continuous tone) and speech audiograms were taken. These measurements were repeated 2 1/2 hours after the glycerin had been consumed, at which time any possible improvement in hearing has usually reached its maximum. A glycerin test was adjudged to be positive if it produced a tone-threshold improvement exceeding 10 dB within at least two octave bands of the measured frequency range, with a simultaneous improvement in discrimination exceeding 14% (in the comparison different lists of test words were used)

The caloric test

The test was made with water at 30 C and 44 C and, if a response was not obtained also with ice-cold water Nystagmography (ENG) was regularly employed (Aschan, Bergstedt & Stahle, 1956). The evaluation of the nystagmograms was based solely on the maximum in

Table I. *Comparison between the caloric response and the hearing based on repeated tests over a long period in 23 cases of Menière s disease*

	Fluctuating hearing loss	Non-fluctuating hearing loss	Total
Normal caloric response	5	2	7
Fluctuating caloric response	4	7	11
Reduced caloric response	3	2	5
Total	12	11	1

tensity (Stahle, 1956 1958) while the durations were left out of account. The percentage difference in excitability between the right and left ear was then calculated (Jongkees & Philipszoon, 1964) Differences exceeding 20% were adjudged to be pathological.

As a criterion of the effect of glycerin on the caloric response we required a difference of at least 10% between the reactions before and 2 $^{1}/_{2}$ hours after the glycerin consumption.

RESULTS

The caloric response during spontaneous fluctuations of the hearing loss

The measurements were made on at least four different occasions over long periods of the disease, varying between 8 months and 8 years (average 4 years) The mean value of the air conduction at frequencies of 500, 1 000 and 2 000 Hz was used as a measure of the hearing loss, which was adjudged to fluctuate or not to fluctuate according to the following criteria. (1) *Fluctuating hearing loss* Spontaneous improvement in hearing exceeding 10 dB observed on at least one test occasion. (2) *Non-fluctuating hearing loss* No improvement in hearing noted.

The caloric response was adjudged to be al (constant) fluctuating or reduced ac rding to the following criteria. (1) *Normal excitability* The difference between the percentage excitabilities of the two labyrinths was equal to or less than 20% (2) *Fluctuating excitability* The differences in percentage excitability exceeded 20% and there was improvement on one or two occasions. (3) *Reduced excitability* The differences in percentage excitability exceeded 20% at least in the last three measurements.

The caloric reaction, remained normal in 7 cases, fluctuated in 11 cases and was reduced in 5 cases (Table I) The hearing loss fluctuated in 12 cases and did not fluctuate in 11 cases. As the table shows, however there is poor agreement between the fluctuations in caloric response and hearing loss. The most common observation, which was made in 7 cases, was a fluctuating caloric response and a non-fluctuating hearing loss. Examples of simultaneous fluctuations in hearing and caloric response are given in Fig. 1

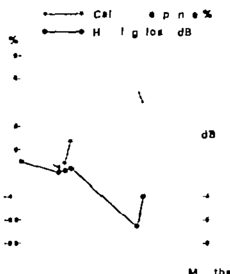


Fig 1 Comparison between repeated caloric tests and hearing tests in a patient with Menière's disease. Examples of simultaneous spontaneous fluctuations in both caloric response and hearing.

The caloric response and hearing after glycerin consumption

As an introduction, the effect of glycerin on 7 normal subjects was studied. The caloric reaction before and after the consumption of glycerin remained unchanged, however and was within normal limits in all cases but one, in which the response had decreased in one ear 2 $^{1}/_{2}$ hours after the consumption. In all subjects the pure-tone audiogram was normal and remained unchanged.

The effects of glycerin on the 32 patients with Menière's disease are summarized in Table II. The differences in right-left sensitivity increased in 11 cases, decreased in 9 cases and remained unchanged in 12 cases. Thus, the distribution between these alternatives was very even and it may be emphasized that the caloric response increased as often as it decreased on the diseased side. A change

Table II. *The effect of glycerin on caloric response and hearing and a comparison between these two factors in 32 patients with Menière's disease*

A positive glycerin test involves an improvement in hearing and this improvement took place in 17 cases. The most common individual combination seems to be an increase in right-left sensitivity (= reduction of the caloric response in the diseased ear), together with a positive glycerin test (= improvement in hearing). The next most common effect is that the patient is not affected by the glycerin, either in the non-acoustic or in the acoustic labyrinth (7 cases).

Caloric response on glycerin administration	Glycerin effect on hearing		
	Positive	Negative	Total
Right-left sensitivity increased	8	3	11
Right-left sensitivity diminished	4	5	9
Right-left sensitivity unchanged	5	7	12
Total	17	15	32

in the caloric reaction in some respect after the consumption of glycerin was thus recorded in altogether 20 of the 32 patients. As regarded the hearing loss, the glycerin test was positive in 17 cases (improvement of hearing) and negative in the other 15 cases, which means that a marked reduction of the hydrops influence could be assumed to have ensued in approximately half the cases.

In a comparison between the caloric response and the hearing loss after glycerin administration, it emerged that no marked combination pattern could be distinguished. The most common combination, however was a positive glycerin test, combined with an increase in the difference in right-left sensitivity through a further reduction of the excitability on the diseased side. This was noted in 8 patients (Table II)

The results reported above argue that *the caloric response may vary independently of the hearing loss* whether this loss fluctuates spontaneously or is greatly reduced by glycerin. If the degree of hydrops is of any importance

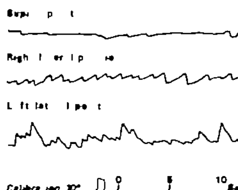


Fig 2 Direction-changing positional nystagmus in normal subject, recorded 2 / hours after the consumption of glycerin. The nystagmus is left-beating in the right lateral position and right-beating in the left lateral position. There is no distinct nystagmus in the supine position.

at all for the vestibular dysfunction, it appears to be at any rate less marked and homogeneous, compared to its effect on hearing.

Positional nystagmus after glycerin consumption

In the positional test regularly performed previous to the caloric test we noticed that glycerin may induce nystagmus in both normal subjects and in patients with Menière's disease. The nystagmus pattern can principally be described as direction-changing (Nylén's type 1) It could be recorded to a more or less marked

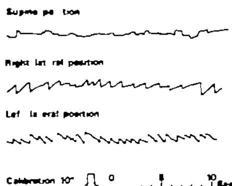


Fig 3 Direction-changing positional nystagmus in patient with Menière's disease on the left side, recorded 2 / hours after the consumption of glycerin. The pattern of the nystagmus is in principle the same as that in Fig. 2.

degree in 6 out of 7 normal subjects and in 14 out of 32 patients with Menière's disease $2\,^1/_2$ hours after the consumption of glycerin. In the most marked cases the nystagmus was left-beating in the right lateral position and right-beating in the left lateral position (Figs. 2, 3) In other cases the nystagmus appeared only in one lateral position or was found in the supine position, only to change direction in one lateral position or the other

DISCUSSION

It has long been well known that the hearing may fluctuate in cases of Menière's disease. Our investigation has shown that the caloric response of the affected ear may also fluctuate. Among 23 patients, who were followed for a considerable time, we found fluctuations in the caloric response in 11 while the hearing fluctuated in 12. However it is noteworthy that these variations seldom coincide in one and the same individual. The opposite state of affairs was more common. This makes it improbable that hydrops has a uniform effect on the two different parts of the inner ear if hydrops influences the non-acoustic function at

view of the known fact that changes ob- ble by light microscopy may be absent the acoustic and non-acoustic neuro-epithelium, it is reasonable to assume that there may be an intralabyrinthine disturbance of a more fundamental nature than hydrops alone and that this disturbance may be of a biochemical nature. It was observed long ago that the consumption of salt might have adverse effects. Carefully controlled drug tests have also shown convincingly that diuretics have a favourable effect, particularly in relation to the attacks of vertigo. In this connection our impression has been that it is rather the amount of the sodium chloride excreted than the general effect of the diuretics that is of value. In this way it is reasonable to imagine that there is some disturbance in the sodium–potassium balance, but in the cases in which it was considered that the difficult sampling and analysis of endolymph could be carried out with an acceptable degree of relevance, no findings were made that differed markedly from the normal (Wullstein & Rauch, 1961) It is therefore possible that we should seek for the cause of the disease also in other factors than a disturbance of the sodium–potassium balance Rauch (1968) has done so and is of the opinion that it is a question "primarily of a disease of the perilymph" According to him, the cause is a capillary dysfunction that arises on a neurovegetative basis, with a concomitant increase of protein in the perilymph and consequently also an increase in the quantity of perilymph. If the Reissner membrane is functioning normally this will result in hydrops. However the question still remains as to the reason for the lack of agreement between the hearing and the caloric response.

The question then arose as to whether glycerin-induced improvements in hearing were accompanied by changes in the caloric response. It became clear that the caloric reaction may be altered after the consumption of glycerin, this was observed in 20 out of a total of 32 patients. However in this connection it should be noted that the caloric reaction was reinforced and reduced on the diseased side in approximately equal numbers of patients. In a comparison between the effects of glycerin on the caloric reaction and the hearing in the individual cases, it also became clear that there was no distinct parallelism in the functional changes. Here it may be noted that the most common individual reaction pattern after glycerin consumption was reduced caloric response on the diseased side, combined with improvement in hearing. Thus, in several patients the glycerin consumption led to opposite functional effects in the two parts of the inner ear

In connection with the glycerin studies, we made a surprising discovery namely that glycerin may induce positional nystagmus in both normal subjects and patients suffering from Menière's disease. In the most marked cases

it was left-beating in the right lateral position and right-beating in the left lateral position, a pattern which corresponds to positional alcoholic nystagmus, phase 2 (Aschan et al., 1956). We do not yet know whether glycerin-induced nystagmus also has a phase 1 like alcoholic nystagmus, but this question will be the subject of further study. The similarity between nystagmus induced by ethyl alcohol and nystagmus induced by glycerin may be due to the fact that glycerin is in principle an alcohol whose pharmacological effects are nevertheless considered to differ from those of ethyl alcohol.

As far as we have been able to discover the effect of glycerin on normal subjects has been little studied. On the basis of our limited material we have found that the subjective reactions are characterized primarily by thirst and headache complaints which may probably be directly assigned to the hypertonic effect of the glycerin on the blood, with secondary osmotically conditioned lowering of the intracranial pressure and changes in the cerebral metabolism. Several experimental subjects also complained of a certain lethargy and a slight feeling of uncertainty as regarded equilibrium.

The location of the trigger point for glycerin-induced positional nystagmus is at present unknown. If we were to draw a parallel with the mode of action of ethyl alcohol, it would be situated in the labyrinth, since a functioning inner ear has been shown to be a prerequisite for the genesis of alcoholic positional nystagmus (Aschan et al., 1956). Our results do not contradict the supposition that this is also the case with glycerin. It became clear that 6 out of 7 normal subjects had positional nystagmus after consuming glycerin but only 14 out of 32 patients. As regards the remaining 18 we may assume that the disease produced such changes in the inner ear that conditions no longer existed under which this form of positional nystagmus could be triggered off. However it may be observed that there is no clear connection between the caloric reaction pattern and the occurrence of positional nystagmus. A heavy reduction in the caloric response does not seem to exclude the genesis of positional nystagmus. This may be interpreted by saying that the functional state of the semicircular canal organs is not of decisive importance, but that the glycerin takes effect via other sensory organs in the labyrinth.

ZUSAMMENFASSUNG

Bei Patienten mit Menière'scher Krankheit kann sich die kalorische Reaktion des erkrankten Labyrinths im Lauf der Krankheit ändern. Schwankungen in der kalorischen Reaktion und im Gehör können unabhängig voneinander auftreten. Nach Verwendung von Glyzerin tritt die kalorische Reaktion des von Menière'scher Krankheit befallenen Ohres geschwächt, unverändert oder verstärkt in Erscheinung. Änderungen in der kalorischen Reaktion und im Gehör wurden miteinander verglichen. Eindeutige individuelle Zusammenhänge konnten nicht festgestellt werden. Mögliche Gründe für die mangelhafte Übereinstimmung in der Reaktion des akustischen und nicht-akustischen Labyrinths, sowohl während der Krankheit als auch nach Verwendung von Glyzerin, werden erörtert. Bei normalen Individuen wurde in einem von sieben Fällen durch Glyzerin die kalorische Reaktion einseitig herabgesetzt. Das Gehör wurde nicht beeinflusst. Glyzerin kann richtungswechselnden Lagenystagmus sowohl bei normalen Individuen als auch bei Patienten mit Menière'scher Krankheit hervorrufen. Diese Beobachtung wird als Antwort des Labyrinths auf Alkohol gedeutet.

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COCHLEAR MORPHOLOGY IN A STRAIN OF THE WALTZING GUINEA PIG

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Abstract. The morphology of the cochlear degenerative process was studied in a strain of the waltzing guinea pig with dominant mode of inheritance. Already at birth, pathological features were found consisting of sensory hair coalescence and cuticular protrusions at the periphery of the hair-cell top. With increasing age the hair cells vacuolized, the plasma membranes ruptured and the cytoplasmic debris was dispelled into the endolymphatic space. The degenerative process started in the third row of the outer hair cells *and on the inner hair cells. Spread occurred* from a locus of predilection at the first and second turns; centripetally to involve all rows of hair cells; longitudinally to comprise all but the extremes of the organ of Corti. The supporting cells degenerated later than and in accordance with the neuroepithelium, finally the spiral ganglion showed depopulation of neurons. Other cochlear structures were morphologically normal. It may be that this endogenous sensory cell degeneration is caused by an intracellular error of metabolism initiated by defect in the genetic constitution.

Morphological investigations on the course of *cochlear degeneration in various forms of* hereditary deafness are possible only in animals. Because of their shorter life span, small animals are especially suitable, as they make it possible to penetrate the mode of inheritance and to obtain a broader basis for physiological and morphological studies within a reasonable time. Consequently since the latter half of the 19th century several investigators have studied the labyrinths of many strains of animals with inherited inner ear defects (Marx, 1926 Grüneberg, 1947 Altmann, 1950 1964 Ormerod, 1960 Deol, 1968) Rodents have been commonly used, owing to their ease of breeding and maintenance.

A strain of the waltzing guinea pig was first described by Ibsen & Risty (1929) Their strain showed a recessive mode of inheritance of the waltzing character (Ibsen & Risty 1929 Ibsen, 1932) The waltzers appeared to be deaf from birth and exhibited an utter loss of vestibular function (Davis et al., 1934 Lurie, 1939 1940 1941 Lurie & Dempsey 1939 Cogan, 1940) Examination by light microscopy showed normal cochlear structures at birth. With increasing age a progressive hair cell loss commenced at the first and second turn, spreading downwards to the round window region and upwards almost to the apex. Random sites of atrophy occurred in the stria vascularis With total loss of the organ of Corti, the spiral ganglion also degenerated. *The ampullae, utricle and saccule* had a normal appearance.

A strain of the waltzing guinea pig, originating from six waltzers received from the National Institutes of Health (USA) in 1961 has been bred systematically in our laboratory since 1966 Genetic analysis showed a dominant Mendelian mode of inheritance with a recessive lethal effect (Ernstson 1970) Furthermore, examinations of the vestibular hair cells revealed characteristic degenerative changes (Ernstson et al. 1969 1970).

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The present study was undertaken to investigate the cochlear morphology in this strain of the waltzing guinea pig.

MATERIAL

For this investigation 75 waltzing guinea pigs were used, their age ranged from a few hours to more than one year

METHODS

For light and electron microscopy the animals were treated according to standard procedure (Ernstson et al., 1969). For scanning electron microscopy osmium-fixed preparations were coated with gold, and then examined and photographed with a Cambridge scanning microscope.

RESULTS

Light Microscopy

The gross anatomy was normal at birth. In the old waltzers, however a complete degeneration of the organ of Corti was found, except in the most apical part (Fig. 1 *A B*) Neonatally the hair cell population appeared normal. How ` a number of hair cells showed slight ab- lities of the sensory hair bundles and of t/cuticular region already at this early age. The sensory hair configuration was somewhat disarranged the cuticula protruded slightly into the endolymphatic space. These changes were first discernible on the outer hair cells of the third row and concomitantly on the inner hair cells the upper part of the first turn and the lower part of the second turn were first affected.

With increasing age these abnormalities spread. centripetally to involve all rows of the hair cells (Fig. 2 *A B*) and longitudinally up- and downwards to comprise but the extreme ends of the organ of Corti. Within about a week hair cell disintegration occurred, starting at the same place as where the hair cell top changes were first observed. This process spread analogously The inner hair cells and the first row of the outer hair cells were the last to degenerate and disappear in any particular region. In 2–3-month-old animals almost all hair cells were missing, except at the extremes of the organ of Corti. After the hair cells had disappeared there was a degeneration of the supporting cells. Waltzers of the same age did not in every case show cochlear degeneration to the same extent. In the individual animal, however the organ of Corti on both sides was similarly affected, with only slight, if any side differences in distribution and severity in the various turns. After total degeneration of hair cells and supporting cells, i.e. at an age of about three months, the spiral-ganglion neurons started to disappear In older animals the entire spiral ganglion was eventually lost.

The stria vascularis appeared normal at birth. With increasing age a moderate patchy atrophy was occasionally seen. This tendency to strial degeneration was not pronounced at any age, nor did it occur at an early stage.

Electron Microscopy

Sensory hairs and hair-cell top

The early changes were confined to the sensory hairs and the hair-cell tops. The latter consisted of protrusions of the cuticula into the endolymphatic space, most pronounced at the periphery of the hair-cell top (Fig. 2 *B*) Early signs of cuticular protrusions into the bases of the sensory hairs were also abundant. These early changes were visible predominantly in the outer hair cells of the third row and in the inner hair cells (Figs. 3 4). They were most consistently found at the upper end of the first turn and in the second turn. There were, however scattered patches of similar abnormal hair cells on the third and fourth turn. With increasing age all the hair cells, with few exceptions, underwent these changes.

The sensory hair degeneration caused a disarrangement of the strictly geometrical pattern of the hair bundle. The hairs coalesced progressively from the bases to the tips. Several distinct stages of this process were observed. The first discernible step was a broadened sensory hair

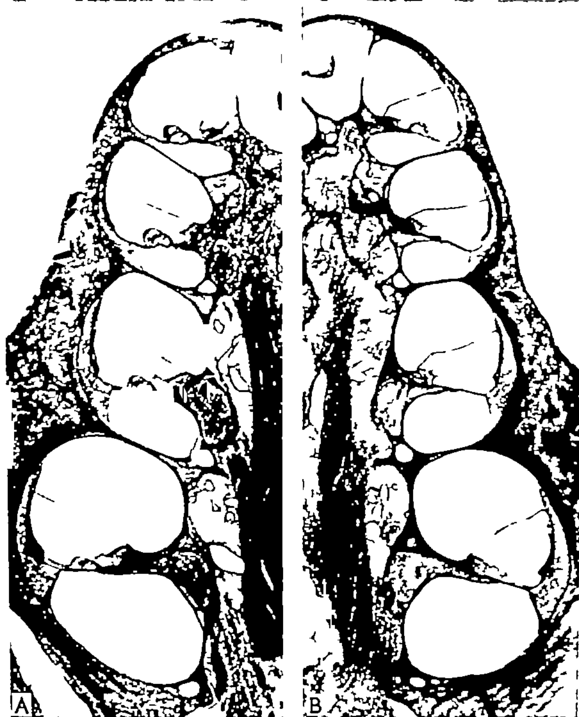


Fig. 1. Montage of two cochleae from two waltzing guinea pigs of different ages. (*A*) From 5-day-old animal. (*B*) From an animal more than 1 year old. Note the normal ppearance of the left half in contrast to the loss of the organ of Corti and the depopulated ganglion spirale in the right half. Other intracochlear structures are largely normal in both animals. Haematoxylin-eosin. (*A*) 373V 42. (*B*) 6V 42.



Fig 2 (*A*) Hair-cell top changes visualized by phase contrast microscopy Defects of sensory hair bundle are evident on the third outer hair cell and on the inner hair cell. 296V 32 days 3rd turn × 1 350 OsO_4-fixation, toluidine blue.

Fig 2 (*B*) Electron microscopy A protrusion (*arrows*) of the cuticular plate into the endolymph was regularly observed as an early sign of patholog. 345V 10 days basal turn. × 5 900.

IHC, Inner Hair Cell, OHC I II III Outer Hair Cells; SH, Sensory Hair DSH Defect Sensory Hair Cu, Cuticula.

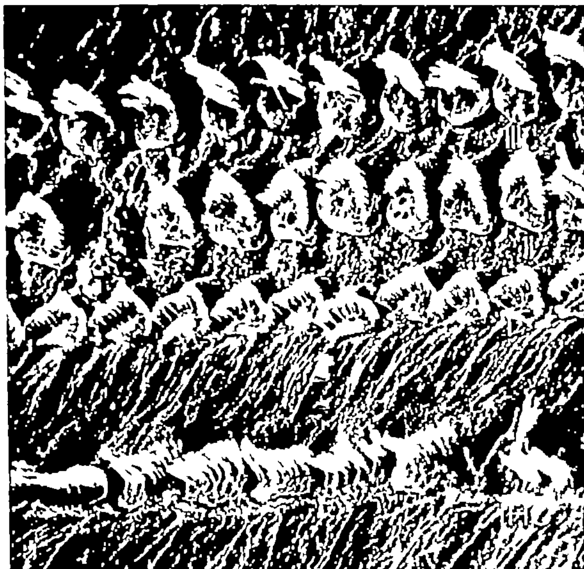


Fig. 3. Scanning microscopy. The disarrangement of the hair bundles of the third (III) row of the outer hair cells is evident. The hair-cell tops are elevated in all the rows, most pronounced in the outer hair cells (OHC) and the third row. Note also the incipient disarrangement of inner hair-cell bundles. One of the OHCs of the second row is either severely degenerated, or perhaps missing (*arrow*). IHC, Inner Hair Cell. 949V 15 days 3rd turn. × 3 600.

base with cuticular substance separating the plasma membrane from the internal fibrillar core (Fig. 5 *A*). There was a narrowing of the space between neighbouring hairs to the point of contact. In a more advanced stage a complete fusion of the basal part of several neighbouring hairs occurred (Fig. 5 *B*). Remnants of plasma membranes were observed within the complexes of coalesced sensory hairs (Fig. 6). In the last stage before hair cell disintegration even the cell cytoplasm with its organelles was found in the grossly deformed sensory hairs (Fig. 7). These changes could often assume grotesque formations before the hair cell was lost.

The sensory hair changes were first seen in



Fig 4 Electron microscopy of hair-cell top. Pronounced protrusion of the cuticula (*black arrows*). The big arrow points toward the broadened sensory hair base. *ESp* Endolymphatic space *IHC* Inner Hair Cell *OHCI* Outer Hair Cell of the first row *ICS* Intracellular Space· *C* Cuticula. Inner hair cell 345V 10 days basal turn 17 000

e same location as the cuticular abnormality ese changes were rather inconspicuous at orth in comparison with the cuticular protrusions. However whereas the cuticular extrusions at the hair cell periphery were non-progressive once they had been established, the sensory hair changes were clearly progressing and interrupted only by the hair cell disintegration.

Malformation of the sensory hairs and hair cell top spread concomitantly centripetally to the second and later to the first row of the outer hair cells, longitudinally towards the apex and down to the round window region.

The plasma membrane finally ruptured and hair cell remnants were expelled into and could be found as debris in the endolymphatic space (Fig. 8).

Cytoplasm and cellular organelles

Neonatally the hair cell cytoplasm and organelles were normal. The first signs of imminent degeneration seemed to be a swelling of the endoplasmatic reticulum. In the later stages of degeneration there was a marked vacuolization of the entire cytoplasm, with the cell boundaries still intact. Interestingly enough, the mitochondria seemed regularly to be the organel

Fig 5 Further progression of hair-cell top degeneration. The sensory hairs are coalesced (*broader arrows*) with several rootlets at the base of the abnormally thick complex (*finer arrows*) The cytoplasm (*CyP*) has protruded into the cuticula. *ESp* Endolymphatic space; *OHC* Outer Hair Cell *I* first row *III* third row (*A*) Outer hair cell 317V 5 days 4th turn 15 300. (*B*) Outer hair cell 184V 4 days 2nd turn 15 000.

ESp
CyP
OHC I
A

ESp
OHC III
B



Fig 6 Inner hair cell, sensory hair coalescence. The arrows point to two rootlets. *Mal* Vesiculated malformation on the sensory hair *ESp* Endolymphatic Space. Crossed arrows demonstrate plasma membrane remnants in the line of fusion between neighbouring hairs. Inner hair cell 296V 32 days 2nd turn × 34 600.

les that were most resistant to vacuolization many of them remained but slightly swollen until the cell remnants were propagated into the endolymphatic space.

Nucleus

The chromatin appeared somewhat more dense than usual. The nucleolus was essentially unchanged. The nuclear membrane was normal and the nuclear pores were approximately normal in number

Synaptic region

The synaptic region was the last part of the cell cytoplasm to be involved in the process of degeneration. Synaptic bars were regularly observed.

Nerve fibres and nerve endings

The afferent and efferent nerve endings in newborn as well as in older animals were apparently normal in number and distribution (Fig. 9) There was no degeneration of nerve

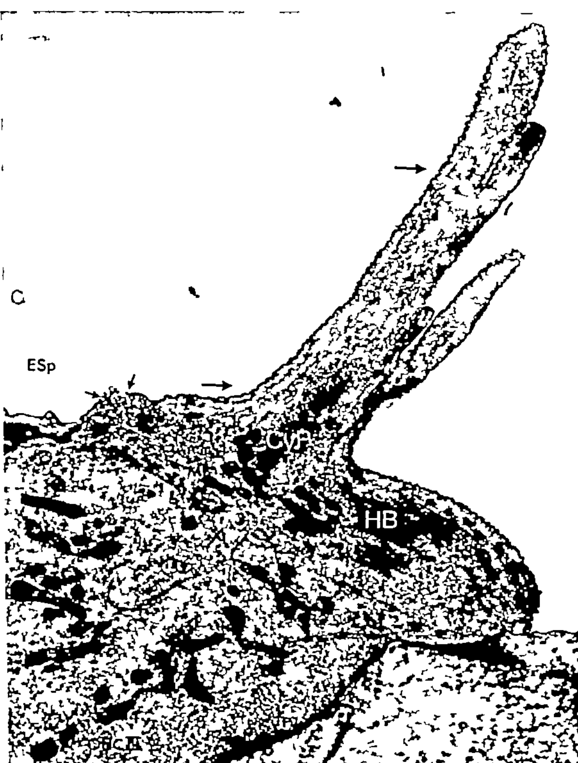


Fig. 7 Grotesque malformation of hair-cell top. Several hairs are coalesced (*broad arrows*). The cytoplasm (*C P*) with mitochondria, multivesiculated body (*MB*) and Hensen body (*HB*), has penetrated above the cuticula (*Cu*). Rupture of the plasma at thin arrows. Outer hair cell 391V turn 13 300.



Fig 8 Further progression of cytoplasmic degeneration of an outer hair cell from the thi d row Through wide defect in the plasma membrane (*arrows*) the oplasmic debris (*CyD*) is xpelled into the endolymphatic space (*ESp*) The only recognizable organelles are the mitochondria (*Mi*) some of them remarkably unchanged. *ICS* Intercellular Space. Outer hair cell, 184V 4 days 2nd turn 13 100.

fibres or nerve endings while the hair cells were present. The radial and longitudinal nerve fibres were apparently normal until the organ of Corti was lost.

Supporting structures

All the supporting cells were of normal appearance until the hair cells had started to disintegrate. The supporting cells then degenerated, following the same pattern as to locus of predilection and spread. The last to disappear were the pillar cells. Finally the whole organ of Corti was replaced by a rather low cuboidal cell layer The tectorial membrane was throughout normal.

Stria vascularis

No regular changes were found In some places the stria was somewhat atrophic, mostly in the very old animals.

Vascular system

No systematic study was undertaken as no apparent anomalies were found at any age.

In short, the progressive changes in the organ of Corti can be described to occur during the first three months of neonatal development and approximized as follows 1–30 days, hair cell top degeneration 10–60 days, hair cell degeneration and disappearance 30–80 days,

Fig. 9 Infranuclear region of an outer hair cell of the second row. The nerve endings are normal, both afferent (*NEaff*) and efferent (*NEeff*). The efferent nerve endings contain several presynaptic dense projections (*blue arrows*). Outer hair cell, 345V 10 days basal turn 33 100.

supporting cell disintegration 90 days and later degeneration of spiral ganglion cells (Fig. 10).

DISCUSSION

Light microscopic examinations of hereditary cochlear degenerative processes have been performed by many investigators. Earlier reports on large materials have shown principally two types of cochlear degeneration. One type is the cochleo-saccular degeneration, first described by Scheibe in two humans (1892, 1895). This type has been found in deaf white cats, in certain strains of dogs, in a strain of white minks and in several mutants of mice (Beyer 1904 Alexander & Tandler 1905 Lagally 1912 Howe, 1935 Wolff 1942 Lurie, 1948 Deol 1954 1956 *a* Wilson & Kane, 1959 Kocher 1960 *a* Hudson & Ruben, 1962 Bosher & Hallpike, 1965 Saunders 1965 Anderson et al., 1968 Sugiura & Hilding, 1970 *a*, *b*). This degeneration is characterized by severe atrophy of the stria vascularis, disappearance of the



Fig. 10 Approximate chronology of the cochlear degenerative process in waltzing guinea pigs with dominant mode of inheritance. ——— denote most intense period of degeneration; signify less active phase *A* Hair-cell top anomaly *B* Hair cell disintegration *C* Supporting cell degeneration *D* Degeneration of the spiral ganglion.

A Ie OI

organ of Corti, deformation of the tectorial membrane adherence of Reissner's membrane to the basilar membrane and the stria vascularis, severe to total loss of spiral ganglion neurons and collapse of the sacculus with atrophy of the macula sacculi. The utricle and the semicircular canals showed in most cases a normal appearance.

The other type of degeneration, the scala media complex (Grüneberg, 1956) has been predominantly found in rodents, such as several mutants of mice, waltzing guinea pigs and waltzing rabbits (e.g. Quix, 1907 v.Lennep 1910 Davis et al 1934 Lurie, 1939 1940 1941 1942 Grüneberg et al. 1940 Grüneberg, 1943 Cogan, 1943 Deol, 1956 *b* Kocher 1960 *b* Mikaelian & Ruben, 1964 Kikuchi & Hilding, 1965 1967) The characteristics of this type of degeneration, as seen under the light microscope, are partial atrophy of the stria vascularis, a total loss of the organ of Corti and a severe to total depopulation of the spiral ganglion No changes have hitherto been reported in the vestibular apparatus

The limitations of light microscopy have been shown by investigations with the electron microscope on shaker I mice by Kikuchi & Hilding (1965) and on deaf Dalmatian dogs by Ander et al. (1968) In shaker 1 the efferent nerve were largely absent and the few efferent fibres that were found appeared later than normal. Furthermore, strial abnormality was not observed until the organ of Corti had degenerated. Similarly no efferent nerve endings were found in the already deaf Dalmatian dogs the afferent nerves were largely normal.

In the present investigation the study by high magnification revealed several interesting features. The abnormalities of the sensory hair and hair-cell top described, have not been observed in the shaker I mice or in the deaf Dalmatian dogs. Furthermore the waltzers had a completely normal afferent and efferent innervation as far as could be judged from the appearance of the synaptic regions

Thus, these waltzers are shown to possess a hitherto unknown type of cochlear degeneration. The progressive deterioration of the hair bundle anatomy and the eruption of the cuticula may suggest a defect in the plasma membrane causing a lack of stability Such a membrane defect might also explain the tendency of the plasma membranes of the sensory hairs to fuse and disappear A genetic defect could conceivably also affect membraneous structures in the hair cell cytoplasm, as for instance, the endoplasmatic reticulum leading to vacuolization and ultimately to disintegration.

It is a matter of further speculation whether both of these manifestations, i.e. of the hair cell top structures and of the cytoplasmatic organelles, are caused simultaneously by a common denominator or one leads to the other At any rate the process is apparently initiated in the hair cell itself, a sequel perhaps, of a minute, but for the hair cell lethal, genetically determined, error of metabolism.

Streptomycin has been shown to cause changes in the sensory hairs of the cochlear hair cells (Duvall & Wersäll, 1964) These induced sensory hair changes were, however never as extensive as in the waltzers nor were there any cuticular protrusions. Furthermore in this exogenous damage the mitochondria were amongst the first organelles to become severely degenerated.

It is interesting to note that genetic cochlear degeneration strictly conforms to the pattern first described by v.Lennep in 1910 The degenerative process seems to be a continuance of the maturative process (Larsell et al 1944 Änggård, 1965) Further studies on cochlear biochemistry may furnish the solution of this problem, as with the electron microscope we are only able to suggest possible explanations to findings as described. For a final analysis I is thus necessary to correlate our morphological findings with further physiological and biochemical data.

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ZUSAMMENFASSUNG

Die Morphologie des Degenerationsvorganges in der Cochlea wurde bei einem erblich dominanten Stamm walzender Meerschweinchen untersucht. Pathologische Veränderungen wurden bereits bei der Geburt gefunden und bestanden in Zusammenschelzung der Sinneshaare und peripheren Auswüchsen der Cuticula. Mit zunehmendem Alter wurden die Haarzellen vakuolisiert, die Plasmamembran riss ein und Zelltrümmer wurden in das Spatium endolymphaticum entleert. Dieser Degenerationsvorgang begann in der dritten Reihe der äusseren Haarzellen und an den inneren Haarzellen und breitete sich aus von einer Prädilektionsstelle in der ersten und zweiten Schneckenwindung: in zentripetaler Richtung alle Reihen der Haarzellen einbegreifend, in longitudinaler Richtung das ganze Corti'sche Organ mit Ausnahme der äussersten Enden. Die Stützzellen degenerierten nach dem Neuroepithel und das Ganglion spirale wies später eine Verarmung an Neuronen auf. Andere Cochlear-Strukturen waren so gut wie unverändert. Möglicherweise wird diese endogene Sinneszellen-Degeneration durch eine intrazelluläre Stoffwechselstörung verursacht.

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GLYCOGEN CONTENT IN THE OUTER HAIR CELLS OF KANGAROO RAT (*D SPECTABILIS*) COCHLEA PRIOR TO AND FOLLOWING AUDITORY STIMULATION

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Abstract Glycogen content in the organ of Corti of the kangaroo rat, *D spectabilis*, is evaluated prior to and following auditory stimulation. PAS positive material is prominent in the outer hair cells, tectorial and basilar membranes, bone, and spiral ganglia. PAS positivity in the outer hair cells is due exclusively to glycogen. Short-term, high intensity stimulation of the kangaroo rats resulted in glycogen diminution in the outer hair cells, accompanied by damage to the organ of Corti in the upper half of the basal turn at frequencies of 1 950 and 3 000 Hz. At 3 000 Hz, the degree of damage increases as the intensity of the stimulus is raised. Long-term exposures at moderately high intensities also show the kangaroo rat to be particularly sensitive to frequencies of 1 950 and 3 000 Hz in the basal and second turns of the cochlea. Glycogen content is diminished, however no cytological damage is apparent.

Assessment of glycogen content in the resting guinea pig cochlea has revealed a high concentration of the polysaccharide in the external hair cells (Zorzoli, 1954 Zorzoli & Borlani, 1958 Falbe Hansen & Thomsen, 1963 Falbe Hansen, 1964 Drucker 1969 Ishii et al., 1969). The demonstration of glycogen in the sensory epithelium acquires theoretical interest due to the fact that the outer hair cells are not vascularized and consequently draw their nutrient and oxygen supply via diffusion. Glycogen storage in the outer hair cells, therefore, may represent a depot to be drawn upon in anoxic periods resulting from functional stress —such as prolonged or intense stimulation—as suggested by Falbe Hansen (1964). Using light microscopy Zorzoli & Borlani (1958) and Vinnikov & Titova (1964) have indeed demonstrated a visual *quantitative decline in* glycogen content of the outer hair cells following acoustic excitation. Recently Ishii et al. (1969) have noted a decrease in the size of glycogen granules in the outer hair cells with the electron microscope in sections prepared from guinea pigs exposed to high intensity white noise.

Prior to, and concurrent with the interest in histochemical changes within the cochlea following stimulation, a number of researchers were, and have become, engrossed in the problem of stimulation deafness and its relationship to structural damage within the organ of Corti (Davis et al., 1935 Smith, 1947 Smith & Wever 1949 Covell, 1953 Engström & Ades, 1960 Spoendlin, 1962) There is general agreement among investigators that the extent of damage to the organ of Corti varies with frequency intensity and duration of exposure. It has also been observed that as the intensity of the stimulus increases, injury is more marked and shifts on the basilar membrane closer to the round window (Covell 1953) such that there is only very limited agreement with the principles of tone localization in the cochlea [e.g. as reported by Schuknecht (1960)]

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The present paper describes results obtained in a histocytochemical investigation of the pattern of glycogen distribution in the cochlea of the banner-tailed kangaroo rat, *D spectabilis* both at rest and following various parameters of auditory stimulation. Except for the recent comparative study of Drucker (1969), research prior to this has centered largely around the guinea pig, a species whose organ of Corti is typically mammalian. The eccentric, modified organ of Corti of the kangaroo rat, *Dipodomys* has been described by Webster (1961 1966) Its auditory sensitivity as determined by peaking of the cochlear microphonic response to pure tones is maximal within a range of 1 000 to 3 000 Hz (Webster 1962)

MATERIALS AND METHODS

Twenty-four male adult, banner tailed kangaroo rats (*Dipodomys spectabilis*) weighing between 100 and 130 g were selected. Before use all animals were individually caged in an animal room kept at 70 F and fed a diet of rolled oats and lettuce.

Prior to stimulation, kangaroo rats were anesthetized with chloral hydrate (0 0076 cc 5% chloral hydrate gram body weight, IP) [illegible] placed on a small raised platform within [illegible] Faraday box, itself located in a sound-[illegible]d room. Sound was supplied to a power driver (Electro-Voice, Model 1829 operated in association with a Model AR150 Reentrant Horn located within the chamber) by an audio oscillator (Hewlett Packard, Model 200AB) used in conjunction with a Dynakit, Mark II Preamplifier Sound intensity was recorded by a calibrated Western Electric, Model 640AA condenser microphone positioned in the sound chamber a couple of inches away from the animal and pointed in the same direction as the animal's external auditory meatus. Measurements taken with the calibrated microphone positioned at various angles within the sound chamber however revealed a remarkable constancy in the dB level at any given frequency variability not exceeding four dB All voltage readings were converted to dB values computed in terms of the calibration of the condenser microphone (0 003V = 74 dB ref. 0 0002 dynes/cm²)

The distribution of glycogen in the kangaroo rat cochleae was evaluated in fixed, paraffin-embedded tissue. Post-stimulation the animals were perfused immediately with 0 85% saline followed by a fixative. Two were employed: a modified aqueous Bouin solution (saturated picric acid, 850 ml. 40% formaldehyde 100 ml. 90% formic acid 5 ml.) and an acidified dichromate-formalin mixture known as Romeis Kaformacet (Kristensen, 1949) Bouin solution effects both fixation and chelation in 2 weeks time while Kaformacet fixes quickly in 3 hours but requires that the tissue be subsequently decalcified after fixation. For this purpose Jenkin's Fluid (Pearse, 1960) was used. Following fixation and chelation the cochleae were washed, dehydrated and double-embedded according to the method of Peterfi (cited by Carleton & Drury 1957) Once embedded, the cochleae were serially sectioned at 10 u and the tissue stained with periodic acid Schiff (PAS) reagent according to McManus (1948). In order to determine what percentage of the stained components of the organ of Corti was glycogen selective digestion of the polysaccharide with malt diastase was carried out on control sections, followed by PAS staining. The tissue was immersed in a solution of 1% malt diastase in 1% saline for 30 min at room temperature according to Pearse (1960) Glycogen distribution along the entire length of the cochlear duct was evaluated with a light microscope and arbitrarily estimated as heavy medium to light, and none Photomicrography was done using a Zeiss Photomicroscope on those sections of particular interest.

In the first series of experiments, kangaroo rats were exposed to frequencies ranging from 500 to 10 000 Hz, at 98 to 131 dB for two to three hours. After stimulation, their cochleae were fixed in Bouin solution and processed according to procedures previously described.

Table I *Experimental series: parameters of stimulation*

Animal no.	Frequency (Hz)	Intensity (dB)	Time (hours)
Series I (Bouin solution)			
DS4148	500	131	2
DS4207A	1 500	131	2
DS4207B	2 000	110	2
DS4192	2 500	110	2
DS4196	3 000	100	2
DS4200	3 000	110	2
DS4176	3 000	121	2
DS4177	6 000	110–115	2
DS4182	10 000	98–100	2
DS4158	Control		
DS4168	Control		
DS4173	Control		
Series II (Kaformacet fixative)			
DS4218	500	120	3
DS4214	750	120	3
DS4215	1 140	120	3
DS4217	1 950	120	3
DS4216	3 000	120	3
DS4211	Control		
DS4 19	Control		
Series III (Kaformacet fixative)			
DS4220	1 950	100	11
DS4221	3 000	98–100	12
DS4222	4 800	98	11
DS4223	7 800	98	11
DS4224	10 000	98	13

The second series of experiments were carried out within a frequency range of 500 to 3 000 Hz, at 120 dB also for approximately 3 hours. These animals however were fixed in Kaformacet after it was discovered in the first series that aqueous Bouin fluid was inconsistent in its preservation of glycogen and thus unsatisfactory for further use. A third group of kangaroo rats was subjected to long periods of stimulation (12 to 13 hours) at lower intensities (98 to 100 dB) and to frequencies in the 1950 to 10 000 Hz range. These animals, unlike those in the first and second series were not anesthetized during stimulation but rather placed in a small wire mesh cage directly beneath the sound source. Anesthesia was administered approximately 20 min prior to perfusion. Detailed parameters of stimulation for the three series are shown in Table I

RESULTS

The distribution of PAS-positive material in serially sectioned cochleae from animals kept in a state of relative quiet was determined. The structures staining most intensely are the tectorial and basilar membranes outer hair cells and bone The spiral ganglia exhibit a variable staining reaction some cell bodies being more intensely stained than others The spiral ligament is moderately stained staining of the stria vascularis is insignificant. Of the six cochleae removed from control animals and fixed in Bouin solution only two revealed a consistent heavy staining of the outer hair cells along the cochlear duct from the upper two-thirds of the basal turn through the lower half of the fourth The thin short hair cells of the lower third of the first turn stain lightly in some animals and show no PAS positivity in others The upper half of the fourth turn is very susceptible to injury during processing, such that some outer hair cells are frequently found missing, and the remaining stain inconsistently The outer hair cells in the other four cochleae exhibit a considerable variability in the intensity of PAS staining, with no particular pattern emergent for any one turn Because of the erratic staining pattern only histological defects were considered to be of significance in the evaluation of results in the first experimental series.

A constant observation which deserves mention, is the apico-basal staining gradient of the hyaline mass within the basilar membrane The basal and second turns are always more intensely stained than the third or fourth This could mean that there is less PAS-positive material in the apical turns or that the entire hyaline mass thins out as it approaches the apex resulting in a quantitative loss of stainable material.

Diastase digestion prior to staining of the cochlear sections proves PAS positivity of the outer hair cells to be due exclusively to glycogen After digestion the external hair cells show a complete loss of stainability as does the



Fig 1 DS 4196 h., 3 000 Hz, 100 dB, 2 hours; organ of Corti, Bouin fixation, PAS. Faint outlines of two rows of outer hair cells (*OHC*) remain, innermost outer hair cell show loss of glycogen, pyknotic nucleus, and irregular cell outline. ×510.

neuroplasm of the spiral ganglion cells. PAS positivity is retained by the tectorial and basilar membranes and bone.

Among the nine experimental animals run in the first series an interesting histological picture emerges in those stimulated for 2 hours at 3 000 Hz at sound pressure levels of 100 110 and 121 dB DS 4196 exposed to an intensity of 100 dB shows spotty histological damage and a decrease in glycogen staining of the outer hair cells in the left cochlea (Fig. 1) On the right side the upper part of the basal turn displays a large area in which glycogen is either diminished or absent. At 110 dB (DS 4200) the two cochleae vary considerably in their histological response to the sound stimulus. On the left side regions of intense positivity alternate with areas rated as moderate or negative while on the right side it could be seen that practically all the outer hair cells in the upper two-thirds of the basal turn are damaged or contain no glycogen. A strong PAS-positive staining is not seen until the upper third and lower part of the fourth turns are reached. Between these two points the outer hair cells contain little or no glycogen. A stimulation intensity of 121 dB produces the most drastic pattern of cochlear damage. On the right side (DS 4176) the upper part of the basal turn displays an area in which the external hair cells are completely missing, the inner hair cells remaining intact. Frequently the cells of Hensen and Deiters cells are gone also (Fig. 2) Immediately above this is a small region, about 180 μm wide, in which the organ of Corti is missing altogether Beyond this point, extending into the lower part of the second turn is an area in which, once again, the inner hair cells remain intact and the outer hair cells are destroyed. There follows a region (340 μm wide) in which the hair cells are not missing but damaged and contain no glycogen. Normal cytology appears rather abruptly in the upper third of the lower half of the second turn, and continues throughout the rest of the cochlear duct. Damage to the left cochlea of this animal is more extensive. The external hair cells of the lower third of the basal turn are injured, the upper two-thirds showing the organ of Corti entirely removed. In the lower half of the second turn the outer hair cells are destroyed, and frequently the cells of Hensen

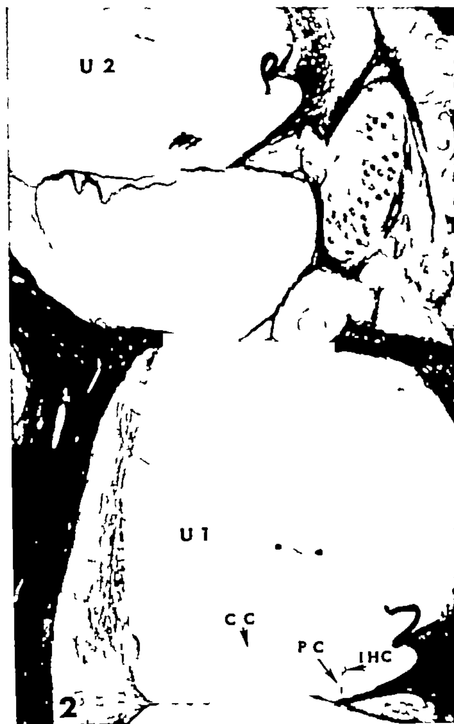


Fig. 2. DS 4176 rt., 3 000 Hz, 121 dB 2 hours, cochlear duct, upper part of basal turn (*U1*) and upper part of second turn (*U2*). Bouin fixation, PAS. Most of the organ of Corti is destroyed in the basal turn, parts of Claudius cells (*CC*), pillar cells (*PC*), and inner hair cell (*IHC*) remain. Normal cytology in second turn. × 190.

are seen rolled up in a ball-like mass in the scala media detached from what remains of Claudius cells. The entire upper part of the second turn and lower half of the third displays damaged outer hair cells. The rest of the cochlear duct is normal

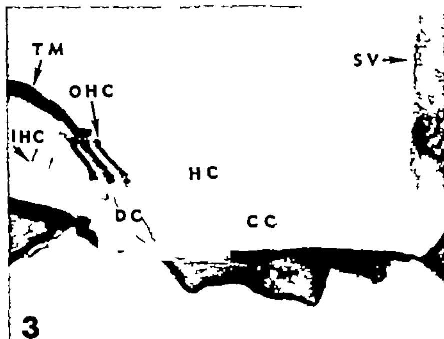
The remaining experimental animals in this first series with Bouin s fixed cochleae show areas in which glycogen appears to be diminished. No injury to the cochleae is seen, nor can any correlation be made between areas of diminution and the stimulus frequency In light of the situation previously mentioned in control animals no significance is attached to these results. For this series, therefore it can be said that, in terms of cochlear damage the kangaroo rats are particularly sensitive to a frequency tone of 3 000 Hz

All animals in the second series were fixed in Kaformacet rather than Bouin solution. Kaformacet was found to be a far superior fixative in the preservation of glycogen in the organ of Corti and in the conservation of cytological detail (Fig. 3) Staining of the outer hair cells is uniformly heavy throughout the cochlear duct, except for the lowermost part f the basal turn where the cells stain moder tely or not at all.

Among the experimental animals, DS 4218 lated at 500 Hz shows no evidence of a crease in glycogen content of the outer hair ells or damage to the sensory epithelium At 50 Hz (DS 4214) the right cochlea displays nly a few outer hair cells with less polysac haride in the very lower part of the second rn the rest of the cochlea appearing normal. he left side resembles a control animal. In ochleae sectioned from DS 4215 stimulated at 1 140 Hz, the middle part of the second turn on the right side shows cells in which the glycogen content has been diminished the left side being unaffected. DS 4217 a kangaroo rat stimulated at 1 950 Hz, is the first in the series to show signs of damage to the sensory epithelium and a decrease in glycogen. Injury to the outer hair cells in the left cochlea is noticed in the entire upper half of the basal turn extending for 870 μm along the basilar membrane. The hair cells which remain are frequently seen shortened (or rounded) with swollen nuclei and devoid of glycogen (Fig. 4). In other areas only cytoplasmic remnants of the cells are left. Occasionally a few outer hair cells are seen which have sustained damage but show PAS positivity At the beginning of the second turn the outer hair cells display a normal cytology but a much lighter staining, indicating that the glycogen content has been diminished (Fig. 5) The rest of the lower half of the second turn is observed to be inconsistently stained from one section to another and within the three rows of outer hair cells in any one section. From the middle of the second turn up to the apex the histology appears normal. The right cochlea of this kangaroo rat is seen to be cytologically similar to the left.

The last member of the series, DS 4216 stimulated at 3 000 Hz, shows a decided dissimilarity between the susceptibility of the right and left cochleae No histological distinction can be made between the right cochlea and an unstimulated control. The left side however exhibits a pattern of glycogen diminution and sensory cell damage very similar to DS 4217 Once again, the area of maximum destruction and glycogen depletion is found to be in the upper half of the basal turn and lower half of the second. In the basal turn, the external hair cells are sometimes seen to be missing altogether (Fig. 6) the remainder showing some degree of injury Almost the entire lower half of the second turn displays hair cells in which the staining is reduced to moderate intensity The exception is a small area, 180 μm wide, near the lower end of the turn, in which the cells maintain a heavy stain.

In the third and last set of experiments, five animals were stimulated for much longer periods of time at moderately high intensities. As noted previously these animals were unanesthetized during the major course of stimulation, anesthesia being administered twenty minutes prior to perfusion.



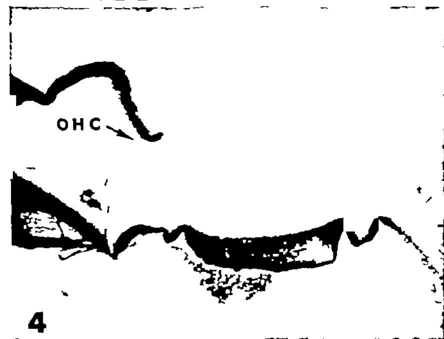
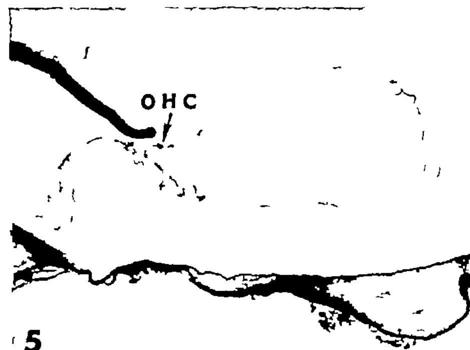


Fig. 3 DS 4211 Control, organ of Corti, upper basal turn, Kaformacet fixation, PAS. *CC* cells of Claudius; *DC* Deiters cells; *HC* Hensen's cells; *IHC*, inner hair cells, *OHC* outer hair cells; *SV* stria vascularis, *TM* tectorial membrane. 400.

Fig. 4 DS 4217 rt., 1 950 Hz, 120 dB 3 hours' organ of Corti, upper part of basal turn; Kaformacet fixation, PAS. Outer hair cells (*OHC*) rounded and devoid of glycogen, nuclei swollen. 410.



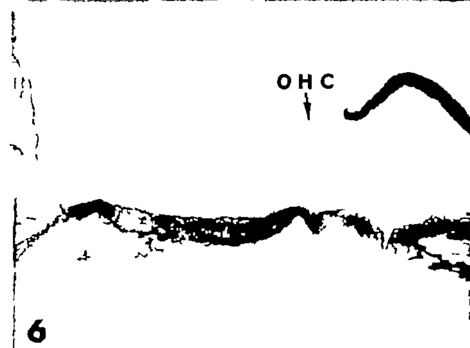


Fig 5 DS 4217 lt., 1 950 Hz, 1?0 dB 3 hours, organ of Corti, lower part of second turn, Kaformacet fixation, PAS. Outer hair cells (*OHC*) are cytologically normal and show a reduction in glycogen content. 4?0.

Fig 6 DS 4216 lt., 3 000 Hz, 120 dB, 3 hours; organ of Corti, upper part of basal turn Kaformacet fixation, PAS. Selective destruction of outer hair cells (*OHC*). 390.

Sections from DS 4222, DS 4223 and DS 4224 are indistinguishable from control animals, i.e. long-term stimulation at 4 800 7 800 and 10 000 Hz has no visible effect on either the general histology or glycogen content. DS 4200 stimulated at 1 950 Hz, is affected on one side only the left cochlea showing a light to moderate staining of the outer hair cells in the second turn. Stimulation at 3 000 Hz (DS 421) also elicited a unilateral response. The left cochlea shows some moderation of glycogen content in the upper part of the basal turn, while the right side is unaffected.

DISCUSSION

The utilization of glycogen in the outer hair cells of the banner-tailed kangaroo rat during sound stimulation is not comparable to that reported by either Zorzoli & Boriani (1958) or Vinnikov & Titova (1964) in the guinea pig, even though the general pattern of glycogen distribution in the resting cochleae is the same. Zorzoli & Boriani (1958) reported a disappearance of glycogen in all the turns of the guinea pig cochlea regardless of tonal frequency if the sound pressure level was kept at 80 dB for periods of 20–30 mins to 4–5 hours. The kangaroo rat requires higher sound pressure levels to lose glycogen in the outer hair cells, and when the loss does occur it is limited to the basal and second turns.

The present research is in partial agreement with Vinnikov & Titova's data showing decrease in glycogen content of the outer hair cells after exposure of guinea pigs to 1 500 Hz, 95 dB for various time periods. This is particularly true in regard to limitation of the effect to the basal and second turns. Rounding of the hair cells and cytological alterations of the nuclei are also comparable, although a higher intensity sound is required to produce the effect in the kangaroo rat. What is not observed, is a conversion of granular glycogen in the external hair cells to a diffuse form, and the establishment of radial and apico-basal gradients of the polysaccharide. Another inconsistency is Vinnikov & Titova's observation that if the frequency of the stimulus is lowered to 300 Hz, cytological changes are centered in the apical turns. Results in the kangaroo rat indicate that lowering the frequency to 500 Hz, and raising the intensity to 120 dB has no visible effect on glycogen content in the cochlea.

The sequence of progressive cytological degradation of the organ of Corti in the kangaroo rat is very much in agreement with that reported by other investigators (e.g. Davis et al., 1935 Suga et al. 1967) the difference being the amount of time and sound pressure level necessary to elicit the response. Davis (1935) was able to produce maximum degeneration of the outer hair cells in the second turn of the guinea pig cochlea at 2 500 Hz, but it required 10–60 days at a sound pressure level of 95–100 dB In the kangaroo rat, similar degeneration was noticeable after stimulation at 120 dB for three hours at either 1 950 or 3 000 Hz, damage being limited to the upper part of the basal and lower section of the second turn.

The relationship of structural changes in the kangaroo rat cochlea to pure tone stimulation at high sound pressure levels coincides well with the recent study of Suga et al. (1967) who studied tonal patterns of cochlear impairment following intense sound stimulation. Using guinea pigs the research measured loss of sensitivity of cochlear potentials in stimulation deafness by use of differential recordings from electrodes implanted in the scala tympani of the basal turn and the scala vestibuli of the third turn. Maximum cochlear potentials for test tones of 500 2 000 and 8 000 Hz at 100 dB were reported to be in the basal turn. It was not until the sound pressure level was dropped to below 60 dB that the measurable response of the third turn overshadowed that of the first at 500 and 2 000 Hz. Subjection of the guinea pigs to 6 000 Hz, for 20 min, at 124 dB reduced sensitivity of the basal turn to these test frequencies, as was indicated by a drop in amplitude of the cochlear potentials.

Furthermore histological examination of the stimulated cochlea routinely revealed loss of the hair cells in the basal turn, slight morphological change in the second, and none in the third and fourth. It would appear therefore that it is the large basal turn which is maximally stimulated at high sound pressure levels (100+ dB) regardless of frequency

The basal turn of the kangaroo rat, however appears to be insensitive to cytological disruption or glycogen loss at frequencies below 1 000 Hz and above 3 000 Hz, even though sound pressure levels are high. The observed susceptibility of the kangaroo rat to glycogen loss and cochlear injury at frequencies between 1 900 and 3 000 Hz, and the animal s comparative insensitivity to frequencies above and below this range, is not surprising in view of Webster s (1962) observations on peaking of the round window cochlear microphonic response in two species of kangaroo rat, *D merriami* and *D spectabilis* Webster reported peaking in amplitude of the cochlear microphonic at 1 400 1 800–2 200 and 2 600 Hz within a frequency test range of 100 to 12 000 Hz. Below 1 200 Hz, the microphonic dropped to within a fifth to an eighth of its peak amplitude above 4 000 Hz, the decrease was even more substantial.

It is almost inconceivable that the large stores of available glycogen in the outer hair cells of the organ of Corti are not more readily consumed when the sensory epithelium is taxed for energy which, as previously noted, would be expected to occur when the cochlea is subjected to high intensity sound or prolonged moderate stimulation. This is particularly true if the oxygen tension drops in the scala media as a result of intense stimulation (Koide et al. 1960) and the hair cells become at least partially dependent upon an anaerobic source of energy production.

It should be pointed out, however that it is not known quantitatively how much glycogen must be utilized by the cells before it becomes visibly apparent using light microscopy It is unlikely that the decrease in size of glycogen granules noticed in the recent EM study of Ishii et al. (1969) after exposure of guinea pigs to white noise for 30 min at 110 dB would be in evidence in a surveillance of stained 10 μm sections with the light microscope. Under the latter conditions, it is quite conceivable that consumption is not noticeable until the cells are on the brink of structural damage particularly since in the present research one condition almost always accompanies the other

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ZUSAMMENFASSUNG

Der Glykogen-Inhalt des Cortischen Organs des *Dipodomys pectabilis* wird vor und nach der akustischen Reizung gemessen. PAS positives Material tritt besonders in den äusseren Haarzellen, tektorial Membranen und basilar Membranen, Knochen und Spiral-Ganglion hervor PAS positives Material in den äusseren Haarzellen rührt ausschliesslich vom Glykogen her Eine kurze, intensive Reizung des *D spectabilis* verursachte Glykogen-Verminderung in den äusseren Haarzellen, zusammen mit einer Beschädigung des Cortischen Organs in der oberen Hälfte der ersten Windung bei Frequenzen von 1 950 und 3 000 Hz. Bei 3 000 Hz steigerte sich der Grad der Beschädigung mit der Erhöhung der Reizungsintensität. Längere Aussetzung bei mässig hoher Intensität zeigt auch, dass die Känguruhratte besonders empfindlich bei Frequenzen von 1 950 und 3 000 Hz in der ersten und zweiten Windung der Schnecke ist. Der Glykogen-Inhalt wird verringert, aber ein cytologischer Schaden ist nicht ersichtlich.

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THE EFFECT OF ETHYL ALCOHOL ON PERMEATION OF ^{24}NA SODIUM INTO THE PERILYMPH

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Abstract. The investigations showed that on injecting a given dose of ethyl alcohol intraperitoneally into guinea-pigs, the permeation process of ^{24}Na sodium isotope into the perilymph is slower and its value is lower than in the vascular bed, in which the activity of the isotope is proportional to the concentration of alcohol in the blood. It was also found that the perilymph activity in those animals to which alcohol was administered was lower than the analogical values in control guinea-pigs.

In view of the widespread consumption of ethyl alcohol and the fact that it is easily absorbed in a practically unchanged form, an attempt to explain the mechanism of its action would seem to be warranted, the more so since spectrum of symptoms which it induces is tensive Among the few and controversial opinions on the effect of exogenic ethanol on the electrolyte balance particularly that of sodium, there is a lack of reports on the effect of ethyl alcohol on the behaviour of sodium in the labyrinthine fluids.

Attempts to apply the results of investigations on animals to human subjects have raised doubts as to the validity of such a comparison. An additional difficulty is encountered in attempts to compare the results of experiments on live and dead animals since it has been found that, even shortly after death, there are significant differences, above all in the composition of the inorganic substances fluids (Maggio 1966 Rauch et al., 1963 Rodgers & Chou, 1966 Silverstein, 1966)

The investigations presented here attempted to answer the question as to whether a single administration of ethyl alcohol to experimental animals would induce disturbances in the permeation of the sodium isotope into the perilymph.

MATERIAL AND METHODS

The investigations were carried out on 53 adult guinea-pigs divided into the following groups:

(I) the control group 13 guinea-pigs to which the isotope ^{24}Na was administered.

(II) the experimental group. 40 guinea-pigs, to which the isotope ^{24}Na was administered together with ethyl alcohol.

In the experiments, perilymph was taken first from one ear and then from the other so that a total of 26 samples were obtained from the control group and 80 from the experimental group After anesthetizing the animals, labelled ^{24}Na in physiological solution sodium chloride was injected intraperitoneally into both groups of animals in doses of 500 μCi/1 kg body weight. The experimental animals also received together with the isotope 10% ethyl alcohol in amounts corresponding to 1 4 g of 100% alcohol per 1 kg body weight. At 30 min intervals, the perilymph was then withdrawn from the tympanic perilymphatic space according to the method of Smith and coworkers (1954) At the same time, blood samp-

Table I *Comparison of the mean rise in activity of ²⁴Na in the perilymph in both groups of guinea-pigs in successive 30 min intervals (imp/min/mg)*

m, mean, S.D standard deviation

	Time (minutes)					
	0–30	31–60	61–90	91 120	121 150	151–180
Control group						
m	43	207	390	455	467	444
S.D.	±29	±120	± 12	± 93	±156	± 68
Experimental group						
m	45	130	182	299	442	360
S.D.	±30	± 10	±120	±120	±110	±115

Table II *Comparison of mean values during the period when there were the greatest differences in the activity of the perilymph and the serum in the control and the experimental group*

m, mean, S.D standard deviation· *p*, probability coefficient

	Activity of the perilymph between 61st and 120th minute of the experiment	Activity of the blood serum between 0–30th minute of the experiment
Control group		
m	420	940
S.D.	± 68	±264
Experimental group		
m	250	382
S.D	±131	± 72
p	0.001	0.001

Table III. *Comparison of the mean rise in activity of ²⁴Na in the blood serum in both groups of guinea-pigs in successive 30 min intervals (imp/min/mg)*

m, mean, S.D standard deviation

	Time (minutes)					
	0–30	31–60	61 90	91 120	121 150	151 180
Control group						
m	940	433	465	405	438	398
S.D.	±264	± 96	±115	± 67	±102	± 45
Experimental group						
m	382	503	425	305	392	320
S.D.	± 72	±140	±139	±121	±117	± 77

Table IV *Concentration of alcohol in the blood of guinea-pigs in successive 30 min intervals (promille)*

	Time (minutes)					
	0–30	31–60	61 90	91 120	121 150	150–180
Concentration (‰)	—	1 79	1.34	1 13	0.77	0.90

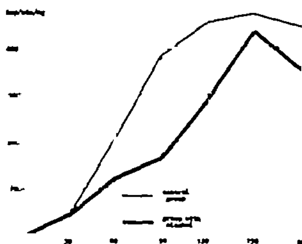


Fig 1 Curves of mean activity of ^{22}Na in the perilymph of control guinea-pigs and guinea-pigs after administration of alcohol according to the time when samples were taken (imp/min/mg).

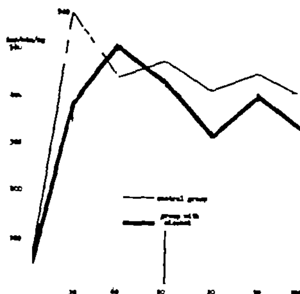


Fig 2 Curves of mean activity of ^{22}Na in the blood serum of control guinea-pigs and guinea-pigs after administration of alcohol according to the time when samples were taken (imp/min/mg)

les were taken from the femoral artery for determination of the activity of the serum and to determine the alcohol content of the blood.

The radioactivity of the perilymph and serum samples was determined by means of a Geiger Müller window counter To each result of the radioactivity a correction was added, in order to take into account the loss in radioactivity as a result of disintegration of the isotope. The number of impulses in 1 min per mg of the investigated fluid was taken as a t of comparison. Alcohol was determined by e Widmark method. The whole experiment, from the moment when the isotope and alcohol were injected to the moment when the last sample was taken, lasted 180 min. The results were analysed statistically by means of the Student formula. From the *t* values the significance of the differences between the investigated series was determined by means of the probability coefficient *p* found in the statistical tables.

RESULTS AND DISCUSSION

The analysis of the results obtained showed that the mean activity of the perilymph samples and that of the serum samples taken at the same time differs significantly in both the control group and the alcoholized animals. The lowest mean activity of the perilymph was observed between the 30th and 60th minute of the experiment whereas the activity of the serum was at that time at its highest (Tables I, III) The highest concentrations of blood alcohol were also observed during this period (Table IV Fig. 3) The activity of the perilymph rises with time and that of the serum falls together with a reduction in the concentration of blood alcohol. The activity curve of the serum samples and the blood alcohol concentration have a similar profile and both show the highest values already in the 1st hour of the experiment (Figs. 2, 3). The curve of the perilymph activity is entirely different, the highest activity not being evident on this curve before the third hour of the experiment (Fig. 1) In the final phase of the experiment between the 121st and 150th and the 180th minute, the mean activity of the perilymph and the serum in both the control animals and the experimental animals, the activity values were similar varying slightly above and below 400 imp/min/mg. The differences occur therefore only in the initial and middle phases of the experiments.

Table V *Comparison of absolute mean rises in activity in the perilymph samples in both groups of guinea-pigs in successive 30 min intervals (imp/min/mg)*

	Time (minutes) ...					
	0–30	31–60	61–90	91 120	121 150	151–180
Control group	43	164	183	65	12	—
Experimental group	45	85	52	117	143	—

On analysing the mean activity of both fluids from the controls and the experimental animals, a higher value was noted in the fluids from the control animals than in those of the experimental animals. In the perilymph the greatest statistically significant differences between both groups occur during the second hour of the experiment ($p = 0\,001$) In the serum the greatest differences occurred within the first 30 min and were also statistically significant: $p = 0\,001$ (Table II)

If one examines the absolute rises in the mean activity of the perilymph samples in the time function of both groups of animals, it can be seen that during the first half of the experiment, that is, up to the 90th minute, there were considerably greater rises in activity and occurring earlier in the controls whereas in the experimental group, under the influence of alcohol, the maximum rises were observed later that is between the 91st and 150th minutes (Table V Fig. 4) In view of the considerable mobility of the sodium ion and the probability of a rapid return to equilibrium owing to the ease with which the cation permeates any kind of biological membrane, in the introductory phase of the experiment perilymph samples were taken a few minutes after the injection of alcohol and the isotope. The activity of these samples was very low The determination of blood alcohol content made at the same time as the above-mentioned determinations showed that the highest concentration, 1 79‰ occurred in the 30th minute. The next examinations revealed a gradual fall in the concentration of ethanol in the blood due to the oxidation of the alcohol in the system as time elapsed (Table IV Fig. 3) The diuretic action of ethanol is well known and for that reason was not determined. Huang & Knoefel (1957) consider that a small amount of ethanol has no effect on the excretion of sodium with the urine. A similar opinion was expressed by Strauss et al. (1960) In this investigation in administering the exactly calculated dose of ethyl alcohol (1 4 g/kg body weight) calculated *per 100% alcohol the fact that this is an* amount that permanently damages the microphonic potential (Giekdanowski, 1965) was taken into consideration. Some kind of ionic shift may therefore be suspected. In view of the possibility of a local effect of ethanol when administered intraperitoneally a 10% dilution was employed in order to reduce to a minimum the local irritation caused by ethanol and the consequences of such irritation (Klingman et al 1958) The high activity of the serum samples noted in the first phase of the experiment would indicate that this aim was at least partially achieved The method for

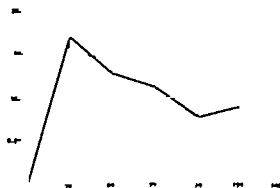


Fig 3 Curves of concentration of alcohol in the blood of guinea-pigs according to the time when samples were taken (promille)

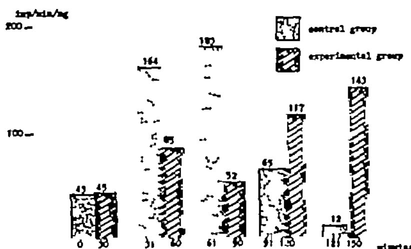


Fig 4 Comparison of absolute mean rises in activity in the perilymph samples in both groups of guinea-pigs according to the time of taking the samples (imp/min/mg).

expressing the activity of the impulses registered in 1 min per 1 mg of the fluid under investigation is of a relative nature but as far as comparison of the various samples is concerned this method gives precise results.

From the results obtained it would appear that ethyl alcohol administered in the concentrations given in this paper damages the mechanism which is responsible for the electrolytic balance, or at least for the balance concerning the sodium ion between the vascular bed and the perilymph. If one accepts the opinion of Schuknecht & El Seifi (1963) and that of auch (1964) that the perilymphatic spaces connected with the cortilymph and that ere is an ionic similarity of the two fluids, it may be assumed that the electrolytic changes observed in the perilymph may also take place in the cortilymph, in which case those functions of the organ of Corti which require a definite concentration of ions would be disturbed The changes in the permeability of the blood–perilymph barrier as regards the sodium ion may suggest that apart from disturbances in diffusion and osmosis, the balance of active transport is disturbed at the level of the semipermeable membranes where sodium plays the role of the activator of certain enzymes in the ATP/ADP system (Whittam, 1964) It is not at present possible to say by what means these changes take place since the action of alcohol covers a very wide range and has a direct effect on the metabolic pathways. The mechanism of these phenomena cannot therefore be interpreted on the basis of these observations and the problem requires further study

On summing up the results of these investigations, it is seen that the highest activity of the perilymph after intraperitoneal injection of the sodium isotope ^{24}Na occurs in the third hour of the experiment, whereas in the serum the highest activity is observed during the first 60 min. This activity is lower and increases more slowly in the group of animals to which ethanol was administered than in control group. The greatest differences in the perilymph activity between the control and the experimental guinea-pigs occur during the second hour of the experiment and are statistically significant.

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurde an Meerschweinchen die nach intraperitonealer Injektion des Natriumisotops ^{24}Na in der Perilymphe auftretende Aktivität und der Einfluss von zusätzlicher Injektion von Äthylalkohol auf diese Aktivität untersucht. Zusammenfassend ergab sich, dass die höchste Aktivität der Perilymphe in der dritten Stunde nach der intraperitonealen Injektion des ^{24}Na vorliegt, während man im Serum die höchste Aktivität bereits i der ersten Stunde beobachtet. Des weiteren ist in der Gruppe der Tiere, die Äthanol erhalten haben, diese Aktivität kleiner und sie steigt langsamer als in der Kontrollgruppe. Die grössten Unterschiede i der Aktivität der Perilymphe zwischen den Tieren der Kontrollgruppe und denen der Experimentalgruppe zeigen sich in der zweiten Stunde des Experiments, und sie sind statistisch signifikant.

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Poland

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SURGERY OF CAROTID BODY TUMORS

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(Received October 26 1970)

Abstract Four cases of carotid body tumor are reported. The value of carotid angiography is stressed, the collection of dye in the tumor being diagnostic. Occlusion of the carotid circulation with clamps during final dissection of the bulb area is recommended for added safety If occlusion takes more than 10 min, use of an internal shunt is preferable. Preoperative planning of the steps to be taken to avoid any possible complications eliminates the hazards otherwise associated with removal of these tumors.

The comparatively rare carotid body tumor develops from the small ovoid carotid body lying in loose areolar tissue in the bifurcation of the common carotid artery This normal structure is considered to be a sensory organ deriving from the glossopharyngeal nerve and ponding primarily to chemical changes with the artery (Engström et al., 1957) This structure has aroused clinical interest recently because of the benefit claimed to result from its removal in patients with bronchial asthma. However the only known pathological condition arising from the carotid body is a tumor consisting of cells similar to those normally present in this structure

The tumor grows slowly and several years may be required for it to reach a size large enough to bring the patient to the doctor The clinical behavior of the tumor is under some dispute and changes suggesting malignancy have been reported in the tumor as well as in the form of local lymph node involvement and distant metastases. Staats et al (1966) in their review reported 3.2 with local and 3.2% with distant metastases among 500 cases. The presence of metastases need not imply any significant alteration in life expectancy An derson & Scarcella (1963) mention a patient who lived for 25 years in spite of distant metastases.

Symptoms

The first sign of carotid body tumor is a lump in the neck. The tumor is painless, transmitting the pulsations of the carotid artery This sign is not diagnostic, being common to all tumors situated in this region. The growing tumor may extend towards the clavicle in spindle-shaped fashion while the large, bulky part grows in cephalad direction The tumor forms a readily noticeable swelling in the submental-subparotid area and may appear as a smooth-walled retrotonsillar enlargement shifting the posterior lateral pharyngeal wall towards the midline.

When the tumor grows upwards it envelops the antero-lateral part of the vagus nerve and encloses the hypoglossal nerve. At the base of the skull the accessory and glossopharyngeal nerves may also become encircled. However in only very advanced cases does paralysis develop and the nerves generally are fully functioning even if encased within the tumor

In diagnosis, the most important thing is to keep in mind the possibility of carotid body tumor Morfit et al. (1953) pointed out that the diagnosis was readily made by inexperi-

enced members of their group who thought of this tumor as one of the first possibilities where as experienced workers were apt to consider first the more common conditions like metastatic lymph nodes, branchiogenic cysts, neurinomas or homolateral thyroid enlargement. Carotid angiography is of great value in diagnosis and has replaced biopsies or aspirations. Angiography as pointed out by Engström & Hamberger (1957) Lowdon (1964) Conley (1965) and Westbury (1967) presents a diffuse mottled collection of dye in this neoplasm and outlines the caliber size and distortions of the main carotid vessels as well as the vascular spaces of the tumor

Surgical Removal

Excision of the carotid body tumor is considered to be the only possible treatment. It demands skill and experience in vascular surgery if grave sequelae are to be avoided. Farr (1964) reported on 37 patients seen at Memorial Hospital (New York): of 21 patients with internal pharyngeal extension, 11 had complete resection, 4 an incomplete resection, and 6 carotid arterial resection or repair 5 of these latter patients died postoperatively Up to 1945 he had resected the carotid bifurcation in 8 patients, with postoperative fatal hemiplegia in 4 or 50% In Farr's subsequent series of 18 carotid body tumors there were three perforations of the bulb with one postoperative death.

Morfit (1965) reported on 21 cases from the period 1950–64 including three resections with transient hemiplegia in 2 cases, and two anastomoses of the external to the internal carotid with transient aphasia in one. Sixteen patients with intact carotid system showed no sequela. Anderson & Scarcella (1963) had 15 patients with carotid body tumor 13 of whom were resected without sequelae. In one case severe hypotension and emergency carotid artery ligation were performed and a graft inserted 1 hour later: the patient became hemiplegic and later died. In the resected cases the internal artery was ligated in 3 and replaced with a graft in another 3 and there were neurological sequelae in none of them. Conley's (1965) series of 29 cases had some type of a vessel rehabilitation in 30% Westbury (1967) reported on 12 cases with one postoperative death and stressed the value of measuring post-occlusion pressure in the internal carotid before resection of the bulb Som et al. (1966) had 8 cases with a fatal outcome in one, and recommended the use of an internal shunt.

Report of Cases

Case 1

This patient was 33 years old and had noticed a lump on the right side of her neck 14 years earlier She had consulted several physicians and one had performed tonsillectomy because of adenopathy" Another physician started antituberculous medication combined with X-ray treatment. The patient then stopped consulting physicians until December 1966, when she was seen at our Out-patient Department.

On the right side of the patient's neck there was a slightly longitudinal tumor 7 to 8 cm across, the right pharyngeal wall bulging almost to the midline. The tumor was hard and non-tender without obvious pulsation, except at the area of the bifurcation. Carotid angiography showed marked distribution of the dye at the area of the tumor (Fig. 1) At surgery the large tumor was found to be adherent to its surroundings and to extend caudally in spindle-shaped fashion, around the common carotid artery Upwards, the tumor extended to the base of the skull. Dissection between the media and adventitia was started around the common carotid artery and continued almost to the bifurcation. The internal carotid artery was found at the postero-lateral border of the tumor and it was slowly dissected free up to the base of the skull. The upper pole of the tumor could not be liberated at this stage. Therefore, dissection was continued at the bulb area with tapes passed round the common and internal carotid. The external carotid artery was freed, ligated and cut, and a good-sized




Fig 1 Carotid angiography in case 1 A large vascular tumor extends halfway towards the clavicle and up to the base of the skull

was then seen to enter the tumor from the bi ation. This artery was cautiously ligated, ich resulted in marked decrease of bleeding rom the tumor The hypoglossal nerve was totally enveloped by the tumor and was freed from it by sharp dissection. The upper pole of the tumor could be liberated by combined blunt and sharp dissection (Fig. 2) keeping the internal carotid protected

Postoperatively the patient had a loss of hypoglossal tone a total right-sided recurrent nerve paralysis, and Horner's syndrome. Initially she had slight difficulties in deglutition but these were rapidly relieved. During follow up examinations there has been no return of hypoglossal function. The recurrent nerve paralysis has remained unchanged but the cord is in a paramedian position and the patient has a good voice.

Case 2

This patient was 41 years of age. She had been under treatment because of right-sided thyroid nodules with some signs of toxicosis. The patient consulted our Out-patient Department on 1 9 1967 with the complaint of a right-sided lump higher up on the neck of 3–4 years duration. She had also had giddiness occasionally and once she had an attack of unconsciousness lasting for some minutes. PBI was 8 7%

At examination a tumor the size of hen's egg was found on the right side of her neck. The swelling was painless, showed clear pulsation and moved easily from side to side. There was also a separate lobular mass slightly lower on the neck, adjoining the mass at the area of bifurcation. Carotid angiography was made, the vessels were found to have normal caliber and,

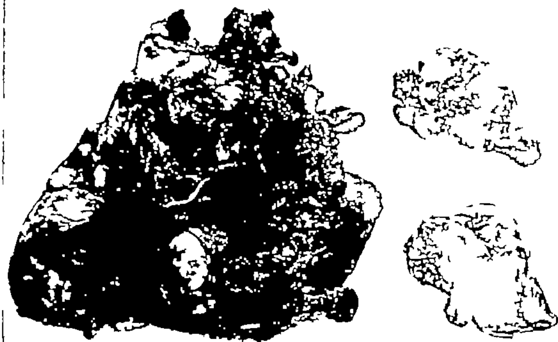


Fig. 2 The removed tumor in case 1. The two separate pieces are parts of the upper pole that had to be removed by combined blunt and sharp dissection. The reduced bloodless tumor measures 5 7 cm.

at the area half way from the clavicle up to the skull base, the mottled pattern caused by the dye indicated a large carotid body tumor.

At surgery several nodules were palpated in the right thyroid and a hemithyroidectomy was made. The common carotid artery was dissected free and a carotid body tumor found, extending 4 cm caudally from the bifurcation and cranially to the proximity of the base of the skull. A tape was passed round the common carotid and, by following the plane of the adventitia and media, the lower pole of the tumor dissected to the bifurcation.

The internal carotid artery was localized at the postero-lateral border of the tumor and the dissection started near the skull base. Another safety tape was passed round it. The hypoglossal and vagus nerve could be dissected intact and freed from the tumor. When the tumor was attached only at the bifurcation, a small rupture of the bulb occurred. The tapes were fastened over the rubber tubes, the tumor was removed quickly and suturing of the rupture started. However a pool of blood suddenly appeared in the wound the blood came from the internal carotid which had been pulled with the tape into the tube so that the two ends were totally separated. An internal shunt was quickly inserted and an end-to-end anastomosis performed in the internal carotid. The carotid bulb laceration was sutured and the shunt recovered from a separate incision on the thicker wall of the common carotid. Heparinization of the artery was made and the wound closed.

Four hours postoperatively evacuation of a hematoma had to be performed. The carotid system was intact and the bleeding came from many small veins in the form of diffuse ooz. Protamin sulfate was given to c.



Fig 3 Carotid angiography 6 months postoperatively in case 6 after end-to-end suture of the internal carotid and repair of the bulb. Arrows point to the internal carotid artery

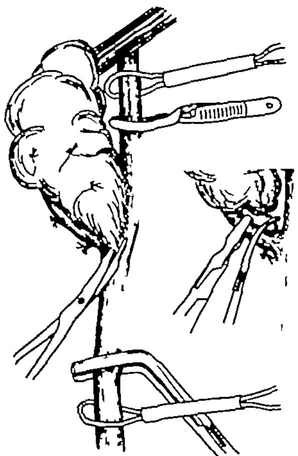


Fig 4 Schematic presentation of the recommended last steps before removal of the tumor An angled DeBakey clamp occludes the common and a small delicate clamp the internal carotid. The tapes are loose. External carotid and the feeding artery are ligated and cut and the tumor removed (*inset*). Possible laceration is sutured.

effect of heparin and the patient recovered without neurological sequelae. During the first 6 months after operation, she once had a sensation of giddiness Angiography was made and it disclosed a patent internal carotid (Fig. 3) The patient has had no complaints since the serum PBI has been 6 6

Case 3

This patient was a 38-year-old farmer who had noticed a slowly growing tumor on the left side of his neck during the last 4 years Two years ago he had consulted a general surgeon, who had attempted a biopsy without success. The patient had some pain behind the ear he felt that the tumor affected his speech and he had some difficulties in moving the head.

The patient consulted our Out-patient Department in October 1969 On the left side, the neck mass was the size of a large hen's egg, it was non-tender and slightly mobile, with clear pulsation on top of the bifurcation. In the mouth, the tumor caused the left tonsil to deviate in the midline Carotid angiography was made and showed a vascular tumor around the bifurcation, corresponding to the area of the neck mass.

At surgery the tumor appeared to surround the common carotid artery as a spindle-shaped extension of the main mass above the bifurca-



Fig. 5. Carotid angiography in neurinoma. The carotid arteries are displaced from their normal course but the tumor shows no vascularization.

tion. The dissection was started between the adventitia and media and continued upwards to the bifurcation. A tape was passed round the common carotid. The vagus was partly enclosed by the tumor but could be dissected free without damage. The hypoglossal nerve ran right into the tumor and was deliberately cut at the posterior border of the tumor. The postero-lateral border of the tumor was slowly liberated from the base of the skull and the internal carotid artery was located in this area. The artery was dissected free at the upper pole of the tumor, a tape passed around it and dissection continued towards the bifurcation. Heparin (50 mg) was then injected into the common carotid artery and an angled DeBakey vascular clamp applied to the common carotid and a small straight one to the internal carotid artery, both tapes being held loose (Fig. 4). In 8 minutes the tumor was removed from the carotid system by ligating and cutting of the external carotid as well as the artery supplying the tumor from the bifurcation. The upper clamp was removed, then the lower clamp, and the common and internal carotids were seen to be intact with good pulsation. The tumor was excised from its anterior attachments and removed. The hypoglossal nerve was freed from the tumor and sutured to the proximal stump.

The patient made an uneventful recovery but had hypoglossal paralysis. Six months later there was some return of hypoglossal function. As the patient continued to have pain in his throat and had chronic tonsillitis, a tonsillectomy was performed in August, 1970.

Case 4

This patient was 65 years of age. She had noticed a slowly growing tumor on the left side of her neck for 5 years. The tumor was

about 4 × 5 cm in size, non-tender without much mobility and there was a strong pulsation The patient also had stenocardial symptoms and was on nitroglycerine and digitalis medication Left carotid angiography was carried out and revealed the typical vascularization of a carotid body tumor The nature of the disease was explained to the patient and a conservative policy recommended. The patient agreed to such treatment and has been regularly followed at the Out patient Department with no noticeable further growth of the tumor

Two other patients have been seen recently with an equally long history of neck mass. On physical examination the neck masses could not be differentiated from carotid body tumor on the basis of the extent of the tumor or findings on palpation. However the carotid angiogram showed abnormal vascularization of tumor in neither case (Fig. 5) and at surgery large neurinomata were removed in each.

COMMENT

These findings in our series of four cases are in agreement with the view advanced by Morfit et al. (1953) that none of the general physi findings are diagnostic in cases of carotid y tumor Consistency pulsation and mobility of these tumors can be similar in a number of other diseases. Carotid angiography as pointed out by Lowdon (1964) Conley (1965) and Westbury (1967) is clearly of decisive help and it should not be omitted when examining tumors of this region A diffuse, mottled collection of dye in the tumor is diagnostic and outlines the tumor area. All necessary preparations for vascular surgery should be made having safety tapes, tapered U 510 Travenol hemodialysis catheters for internal shunts, and vascular clamps of varying size available

The ease of dissection in the layer between the media and adventitia of the carotid is well-established and this line should always be followed. The tumor does not involve the media and can generally be peeled off from the vessel without much difficulty Conley's (1965) suggestion that the internal carotid is to be located at the postero-lateral border of the tumor is well founded Its dissection should always be started in this area, and if the tumor is not very extensive, freeing of the artery can be begun at its upper pole In cases with extensive tumors encroaching on the skull base, the internal carotid should be approached well above the bifurcation but definitely below the skull base. Dissection should then continue upward and the artery be liberated from the tumor even when the latter still remains attached to the skull base. With the safety tapes passed round the arteries, liberation of the internal carotid can be continued towards the bifurcation

Having experienced the complications in case 2, we carefully analysed the possible causes of these mishaps. As a result, we now fasten a tape round the internal carotid only if there is an internal shunt, or otherwise the sharp edges of the tube can cut the artery for instance when held taut by an inexperienced assistant. Without shunt, the internal carotid is occluded with a small clamp only and no traction is possible. The common carotid tolerates the safety tape well, even though pulled unnecessarily taut.

Another thing that we consider an improvement is to have a bloodless field when dissecting the carotid bulb area. It is a well-established fact, in vascular surgery of the neck, that the carotid circulation can be discontinued for 10 minutes without problems, if only the starting arterial pressure is kept at normal or above normal levels. Therefore, we think that the procedure of choice is to effect the final separation of the tumor from the bulb, the weakest area and the area harboring the supplying artery with clamps applied to both the common and the internal carotid artery Excision of the tumor can be made by ligating first the external carotid, and then the supplying vessel. If a small rupture of the bulb occurs at this stage, there is still time to suture it without haste and to remove the clamps. If the

procedure for some reason takes more than 10 minutes, an internal shunt can be introduced and all the reparative work made with an internal shunt in place.

ZUSAMMENFASSUNG

Man berichtet über 4 Fälle von Tumoren des Glomus caroticum, bei denen eine arteriographische Untersuchung deutlich die Spreizung des Farbstoffes in den Tumorgefässen darstellt. Die Autoren empfehlen bei Resektionen dieser Tumoren eine kurze Blokkierung des Kreislaufes als eine gute Sicherheitsmethode. Falls die Blockierung mehr als 10 Minuten dauert, sollte man ein intraarterielles Shunt anwenden. Eine genaue präoperative Vorbereitung um alle möglichen Komplikationen entweder zu vermeiden oder zu reparieren, ist eine Voraussetzung für die Sicherheit der Patienten.

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CILIARY ACTIVITY RECORDED BY TV MONITOR AND PHOTOTUBE

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(Received November 12, 1970)

Abstract A method for recording light-intensity variations obtained from surface-reflexes of living ciliary cells and reproduced on a TV-screen is described. The light-reflexes are amplified using a phototube and an oscilloscope and result in no loss of information. The method described here fulfils the requirements for the greatest possible exactness in reproducing light-variations.

A survey of different methods for studying ciliary frequency and rhythm has recently been published by Chevance & Lennon (1970) They have also performed experiments for the comparison of cilia from different animals and different organs, employing (1) stroboscopic (2) graphic, and (3) microphoto-oscillographic methods They drew the conclusion that the latter method is especially suitable for pharmacodynamic studies It implies, however that one single cilium isolated from its muscular bed and cellular connection is examined through the immersion objective of a phase contrast microscope. Håkansson & Toremalm (1965) have earlier described an examination technique differing from those mentioned above. As in the cinematographic method, it is based upon the variations of light reflexes on the mucous membrane surface brought about by ciliary activity but the opticmicroscopic procedure is carried out with an electronic magnification via a television set and a phototube This method enables registering not only of rhythm and frequency but also the integrated contractions of the smooth muscle layer beneath the epithelium

The present investigation introduces a further technical improvement of this method showing that the light-pulses obtained from the TV-screen can be presented as a curve with continuous variations.

METHOD

Light-spots on a TV-screen are confluent for the human eye, but when recorded electronically by a rapid phototube, each spot is shown to be composed of spike-curves repeatedly appearing with the synchronous frequency of the TV Variations in light-intensity cause variation in the amplitude of the spike-pulses. For practical purposes, light variations can be more simply described as a curve than as spike-height variations. An integrating circuit is therefore desirable.

A solution of this problem has earlier been described (Håkansson & Toremalm, 1965) but in the light of modern electronics it must now be considered as outdated. The use of modern "sample and hold"-technique gives a new solution for this problem.

The block diagram is shown in Fig. 1 Light reflexes appear as twinkling spots on the TV monitor and one end of a thin light wire is therefore placed on such a spot by means of a

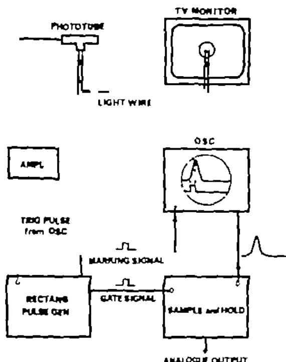


Fig 1 Block diagram of the recording arrangement.



Fig 2 Upper curve: Light from the TV-monitor recorded as a pulse. Lower curve: Marking pulse, duration 0.2 msec, showing sampling-position and sampling-time.

suction-holder attached to the screen of the monitor. The other end of the light wire is connected to a gas phototube. The pulses from the TV are amplified and fed into a doublebeam oscilloscope. The beams are triggered by the synchronous pulses directly from the TV.

A triggering pulse is fed from the oscilloscope into a rectangular pulse-generator (DISA Multistim, Copenhagen)—an ordinary stimulator for laboratory use—which is capable of delivering delayable and coincident marking and stimulating pulses. The marking pulses are fed back into the oscilloscope and are used to verify the time during which sampling is desired. The sampling-time can be placed at will on the curve from the phototube by varying the delay-knob of the stimulator. Fig. 2 shows the gate or marking-signal in relation to the spike-pulses. The other rectangular pulses of the stimulator are employed for opening the gate of the sample-and-hold circuit (Burr-Brown 4034/25). From the output of the oscilloscope the phototube signal is fed into the sample-and-hold module as input signal.

RESULTS

A typical performance gives the signal on the oscilloscope screen as shown in Fig. 2. The upper curve represents one single pulse of about 5 msec duration when a suitable gas phototube has been selected.

Sampling is preset to occur for 0.2 msec during the increasing phase of the signal, as near the peak as possible (the lower curve in Fig. 2). This amplitude is then maintained until the next sampling-period.

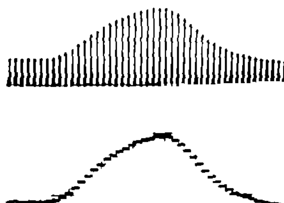


Fig 3 Upper curve: Light-intensity variations on the TV-monitor as recorded by the phototube oscilloscope screen. The pulses have 50 Hz. Amplitude in arbitrary units. Curve of light-variations and-hold technique.

Constant light intensity recorded by the phototube (the upper signals, left, in Fig. 3) gives a constant output voltage from the sample and-hold circuit (Fig. 3 lower left) Variations in light intensity give the integrated voltage-curve shown in Fig. 3

For a synchronous pulse-frequency of 50 Hz, the upper limit of recording without phase interference is 25 Hz.

DISCUSSION

There is an obvious need for simpler and more reliable methods for functional studies of cilia covering mucous membranes, which would enable serious studies of such important clinical problems as the local effects of inhaled toxic gases, pathophysiological changes following infections in the respiratory tract, and the side effects of irradiation therapy

An ideal method for routine pharmacodynamic studies of cilia should be facile in use reliable in running and permit in vivo as well as in vitro studies of an area large enough for investigating the interaction of adjacent cells. The method presented here implies an improvement of earlier techniques. The registration of ciliary beat-frequencies is more objective than in earlier methods, and the experimental results obtained are easily reproducible. Series of experiments investigating specific questions of different types will be published elsewhere.

ZUSAMMENFASSUNG

Eine Methode zur Darstellung der Lichtintensitätsschwankungen durch Oberflächenreflexe von lebenden Ziliarzellen und Reproduktion dieser Reflexe auf Fernsehbildschirmen wurde beschrieben. Die Lichtreflexe werden mit lichtempfindlichen elektronischen *Röhren und einem Oszilloskop ohne Informations*verlust verstärkt. Die hier beschriebene Methode erfüllt alle Anforderungen grösster Genauigkeit bei Registrierung der Lichtschwankungen.

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MINUTES OF AN EXTRA-ORDINARY MEETING OF THE BARÁNY SOCIETY AT THE HILTON HOTEL IN AMSTERDAM ON 8 JUNE 1970

Members present: 33

Invited participants: 37

1

Professor Jongkees welcomed the participants to the meeting.

2

Professor Engström, the President of the Society thanked the Dutch organizing committee, on behalf of all the participants, for their invitation to Amsterdam and Utrecht and recalled the great contributions which Dutch scientists had made in otoneurological research.

3

His Excellency K.-G. Lagerfeldt, the Swedish Ambassador to The Netherlands, gave a survey of the lively cultural relations between The Netherlands and Sweden in his address of welcome and reminded the participants of Robert Bárány's life work. The Ambassador then declared *the meeting open.*

The official opening of the meeting was followed immediately by the *administrative session* under the chairmanship of Professor Groen.

THE ADMINISTRATIVE SESSION

1

Professor Groen declared the administrative session open.

2

Professor Engström proposed four subjects for discussion: (*a*) new members, (*b*) the publication of the transactions, (*c*) future meetings, and (*d*) any further problems.

3

The forms for the election of new members to the Society were discussed. Dr Fredrickson proposed that a committee should be selected which would elect new members. Dr Kornhuber called attention to the fact that in 1968 we selected a committee (Engström, Jongkees, Arslan and Graybiel) which was to scrutinize applications for membership and to make recommendations. However, in 1968 no decision was reached as to when elections should be held—continuously, once a year, or every five years. Professor Dohlman thought that it should be possible for elections to take place immediately. Dr Kornhuber proposed that a new election committee should be selected at each meeting of the Society. Engström, Jongkees, Graybiel and Morimoto were proposed as members of a new election committee. On account of shortage of time, the selection of the committee was postponed until the 10th of June in connection with the meeting in Utrecht.

At the meeting in Utrecht it was decided that Engström, Fredrickson, Hallpike, Ledoux and Morimoto should be appointed members of the election committee and that a new
should be chosen at meeting.

With
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kees stated that it would probably cost about 15 000 Dutch florins. The Dutch organizing committee undertook to publish the transactions in the best possible way. Discussion had been taken up with several editorial boards. Dr Kornhuber proposed that the Society should publish its own official journal. He declared that he was willing to negotiate with Springer Verlag about their cooperating in the publication. Dr Pfaltz proposed that an approach should be made to Karger Verlag and he was willing to negotiate with them on the matter. Dr Henriksson considered the *Acta Otolaryngologica* should be the first choice for the Society's transactions. No decision was made, but Drs Kornhuber, Pfaltz, Morimoto and Fredrickson were commissioned to investigate the possibilities of publishing the transactions in some existing journal and to report to the next meeting.

5

As regards the next meeting, Dr Fredrickson requested that it should be held in Toronto in 1971. The Society decided to entrust Dr Fredrickson with the arrangements for the next meeting in Toronto in August 1971.

Dr Hallpike suggested that a meeting should be held in London in 1972, Dr Greiner a meeting in Strasbourg in 1972 and Dr Pulec a meeting in Los Angeles in 1972 or alternatively 1974. It was decided to postpone the decision about the time and the place of the meetings in 1972 and thereafter until the next meeting in Toronto in 1971.

6

The chairman declared the administrative session closed.

Amsterdam, 10 June 1970

Hans Engström
President

Jan Stahle
Secretary



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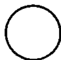
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